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## *Vojnosanitetski pregled*

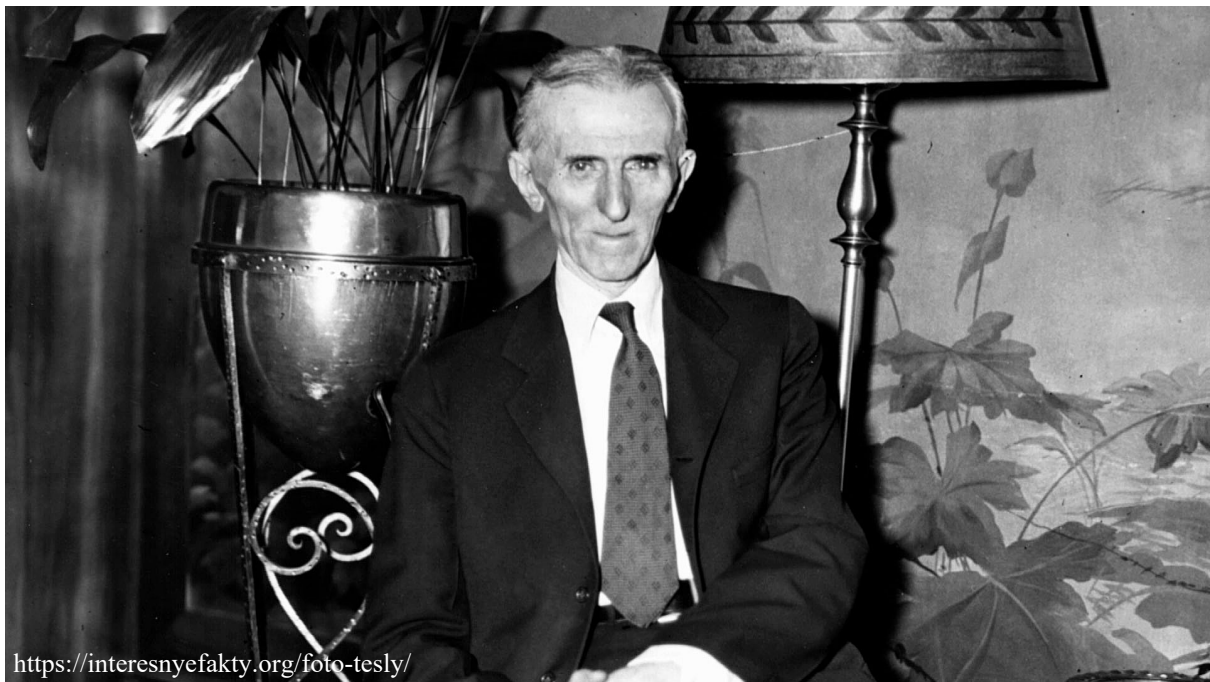
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Vojnosanitetski Pregled



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# VOJNOSANITETSKI PREGLED

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The Journal continues the tradition of *Vojno-sanitetski glasnik* which was published between 1930 and 1941

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# VOJNOSANITETSKI PREGLED

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On January 7th, 2023, 80 years have passed since the death of Nikola Tesla (1856–1943), the last Renaissance figure of the modern era. Although Tesla was not primarily dedicated to biomedical research, his work significantly contributed to the development of radiology and high-frequency electrotherapy (Tesla currents). He is famous for his extensive experiments with mechanical vibrations and resonance, examining their effects on the organism and pioneering their use for medical purposes. Nikola Tesla was the forerunner in discovering electrons, X-rays, radar, electronic microscope, cosmic radiation, and induced radioactivity. It is less known that Tesla's inventions (Tesla coil and wireless remote control) are widely used in modern medical equipment, as well as in numerous diagnostic and therapeutic procedures.

Sedmog januara 2023. godine navršeno je 80 godina od smrti Nikole Tesle (1856–1943), poslednje renesansne ličnosti modernog doba. Premda Tesla nije primarno bio posvećen biomedicinskim istraživanjima, njegov rad je značajno doprineo radiologiji i visokofrekventnoj elektroterapiji (Tesline struje). Ispitivao je i uticaj mehaničkih vibracija i rezonancije na organizam i prvi ih primenio u medicinske svrhe. Bio je preteča otkriću elektrona, rendgenskog zračenja, radara, elektronskog mikroskopa, kosmičkog zračenja i indukovane radioaktivnosti. Malo je poznato da su Teslini pronalasci (Teslin kalem i sistem za bežično daljinsko upravljanje) nezaobilazni deo korišćenja medicinske opreme, kao i brojnih dijagnostičkih i terapijskih procedura.



## The *Vojnosanitetski pregled* in 2022 – Challenges as an incentive for improvement

Vojnosanitetski pregled u 2022. godini – Izazovi kao podsticaj za napredak

Dragana Vučević

University of Defence, Faculty of Medicine of the Military Medical Academy, Center for  
Medical Scientific Information, Belgrade, Serbia

As the Editor-in-Chief of *Vojnosanitetski pregled* (VSP), I have the distinct honor of wishing a warm welcome to the readers of the first issue of 2023. Being in this position in these challenging and unpredictable times is a big responsibility. I would like to thank our former editors, Colonel Professor Tihomir Ilić and Professor Silva Dobrić, for their help in ensuring a successful Editorial transition. In particular, I wish to express my deepest gratitude to Professor Silva Dobrić, who held the position of VSP Editor-in-Chief from 2006 to 2020, for her dedication to the Journal during the last two years. Her precious pieces of advice and engagement facilitated my initial steps in the responsible path of leading a scientific journal. I am convinced that Professor Dobrić will continue to enhance the quality of the Journal through her contribution to the Editorial Board (EB) of VSP.

We were hoping that 2022 would be better than 2021. However, for most of the world, it was another year in pandemic mode, affecting all of us. Besides, the world faced the consequences of climate change and war in Europe with no end in sight.

For VSP Editorial Office (EO) members, last year will be a year to remember. We will recall it by obstacles that seemed insuperable. Some of us will remember it by personal probations and professional challenges that represent a turning point in every person's life. Nevertheless, we will also remember it by the spirit and energy that kept us going and gave us the strength to endure. Thanks to the tremendous commitment and professional responsibility, but most of all, teamwork, VSP EO members managed to fulfill all stages of receipt, review, and preparation of manuscripts without delay, ensuring that our readers and authors were not affected in any way. Therefore, I would like to thank all the members of VSP EO who worked hard every day to achieve these goals.

Furthermore, I deeply appreciate the support received from the members of VSP EB in these difficult times. I would like to express my sincere respect to them.

Sadly, in 2022 we said farewell to Professor Zoran Krivokapić, a world-renowned colorectal surgeon who joined VSP EB already with a reputation as a world-class. We were honored to have him as a member of EB of VSP from 2021 to 2022.

The beginning of the New Year is an excellent opportunity to look back and summarize what we did well.

VSP gladly receives submissions of articles reporting research results related to clinical and experimental investigations obtained in the fields of medicine, dentistry, and pharmacy, thus covering a wide range of topics. During the last year, VSP received insignificantly fewer manuscripts to be considered for publication compared to 2021, 264 vs. 278. As usual, the largest number was from the categories "Original articles" (187 or 70.6%) and "Case reports" (45 or 17%) (Table 1). In the pre-review phase, we declined 134 manuscripts, and 130 (49.2%) entered the review stage. Among reviewed manuscripts, until January 01, 2023, we accepted 37.7% for publication in electronic form (as online first with an assigned DOI number) and rejected 29.2%. The rest (33%) are currently in the review process. We are especially proud of foreign authors for submitting their manuscripts from 18 countries not only from our region but also from all around the world: Australia, Bulgaria, China, Cyprus, Great Britain, Iran, Iraq, Israel, Italy, Mongolia, Montenegro, North Macedonia, Russia, Saudi Arabia, Slovenia, Turkey, and the United States of America (in alphabetical order). Thank you for your trust.

The total number of articles with assigned DOI numbers in 2022 (including the ones submitted for consideration before 2022) was 97. They will be published in printing form in future issues of the Journal.

**Table 1**  
**Categories and the number of manuscripts submitted to the *Vojnosanitetski Pregled* in 2022**

Category	Manuscripts
	n (%)
Original articles	187 (70.6)
Case reports	45 (17)
Current topics	7 (2.6)
General review	16 (6)
Short communications	1 (0.4)
History of medicine	4 (1.5)
Letter to the editor	0 (0)
Editorial	1 (0.4)
Practical advice for physicians	0 (0)
Meta-analysis	3 (1.1)
Book review	0 (0)
Preliminary report	1 (0.4)
<b>Total</b>	<b>265 (100)</b>

We are extremely proud of how we managed to accelerate the editorial review process. In 2022, the average time from submission to first decision was 55 days, and from submission to online ahead-of-print publication was 181 days.

The structure of the papers published in 2022 (Table 2) and the representation of authors, domestic vs. foreign, did not change significantly compared to 2021. Most of the published papers were from the categories “Original articles” (119 or 66.1%) and “Case reports” (39 or 21.7%). Domestic authors made up the majority of the published papers (141 or 78.3%). Of these, 76.11% and 23.8% were written by authors from the so-called civilian health and academic institutions and the University of Defence in Belgrade, Serbia, respectively. The authors of the remaining 21.7% of the published manuscripts were foreign authors from 16 countries from the region and the world. Once again, I would like to express my gratitude to all the authors who chose our Journal to present their results, especially the foreign ones who significantly contributed to the development of VSP and

**Table 2**  
**Categories and the number of articles published in the *Vojnosanitetski Pregled* in 2022**

Category	Articles
	n (%)
Editorial	1 (0.6)
Original articles	119 (66.1)
Case reports	39 (21.7)
General review	1 (0.6)
Current topic	4 (2.2)
Short communications	6 (3.3)
Preliminary report	0 (0)
Practical advice for physicians	3 (1.7)
History of medicine	3 (1.7)
Letter to the editor	3 (1.7)
Book review	0 (0)
Mini review	1 (0.6)
<b>Total</b>	<b>180 (100)</b>

stimulated its impact in the scientific and academic community.

Since last year, the Journal’s impact factor has reached a value of 0.245 as a result of all the interesting papers we published in 2021.

According to the Serbian Citation Index, a database through which articles from the VSP can be downloaded in full text, in 2022 (until January 01, 2023), the number of total worldwide visits to the VSP journal homepages was about 1,830, and the number of full-text downloads of the VSP articles is about 51,828. According to the EBSCO database, the number of downloads of the VSP articles published in 2022 was 4,956. In total, 9 articles were downloaded more than 100 times (Table 3), and 56 articles were downloaded more than 20 times. The highest number of downloads of an article is 895, and the title of that article is *Anxiety and depressive symptomatology among children and adolescents exposed to the COVID-19 pandemic - A systematic review*. In total, 3,000 different universities, colleges, libraries, and other institutions from all over the world accessed the papers published in VSP in 2022. That encourages us to continue our editorial policy in 2023. We hope that, in the future, we will be able to continue publishing innovative and important papers for all our readers.

Apart from the unquestionable authors’ contribution, it is essential to emphasize that we would not be able to ensure the scientific quality and produce the Journal without the dedicated support of our EB members and reviewers.

The experts who provided reviews of papers during 2022 are cited in Table 4. I am incredibly grateful to all our reviewers who have voluntarily dedicated their time and expertise to ensure the publication of only the best of the best in VSP. Their highly competent and thoughtful comments were and will remain outstanding support for the Journal.

The beginning of the New Year was a good opportunity to implement changes we can influence to ensure that the ascending path of VSP continues this year. The biggest tasks ahead of us are shortening the time for publication of accepted papers, changing the Journal website and Instructions for authors, and converting VSP to a digital-only issue.

Last year was full of challenges, but it showed us that challenges could also turn into opportunities to make a difference. The greatest challenge this year will be the workload that awaits us. However, remembering everything we did in 2022, I believe nothing is unachievable with teamwork. For our initiatives to become a reality, we receive great support from the publisher’s advisory board of VSP for the benefit of all our readers, authors, and the academic community. Therefore, I am enormously grateful for their understanding.

I hope 2023 will be the year to remember with pride and joy and that we will be able to make the changes my predecessors have considered. Looking ahead, I hope VSP will continue ascending toward the position where the most valued journals of biomedical science are. I sincerely hope this year will be better than the previous one for all contributors to VSP and all our valued staff members.

May the New Year bring lots of health, success, and many beautiful moments to You and Your loved ones.

**Table 3****Articles downloaded more than 100 times, arranged according to their number of downloads (N)**

Article title	Corresponding author	N
Anxiety and depressive symptomatology among children and adolescents exposed to the COVID-19 pandemic – A systematic review (DOI: <a href="https://doi.org/10.2298/VSP210521092D">https://doi.org/10.2298/VSP210521092D</a> )	Sanja K. Djurdjević	895
Children and youth in the institutional setting – mental health characteristics of children from the Center for Protection of Infants, Children, and Youth in Belgrade (DOI: <a href="https://doi.org/10.2298/VSP191205092M">https://doi.org/10.2298/VSP191205092M</a> )	Ivana Milosavljević-Djukić	340
Inflammatory cardiovascular risk markers and silent myocardial ischemia in type 2 diabetic patients (DOI: <a href="https://doi.org/10.2298/VSP201012010M">https://doi.org/10.2298/VSP201012010M</a> )	Gabrijela Malešević	276
Selection of the optimal medical waste incineration facility location: A challenge of medical waste risk management (DOI: <a href="https://doi.org/10.2298/VSP200521072S">https://doi.org/10.2298/VSP200521072S</a> )	Nataša Petrović	142
COVID-19's impact on radiotherapy in the Republic of Srpska (DOI: <a href="https://doi.org/10.2298/VSP211018024K">https://doi.org/10.2298/VSP211018024K</a> )	Goran Kolarević	118
Effect of aerobic exercise on frequency of vaginal birth – A meta-analysis (DOI: <a href="https://doi.org/10.2298/VSP200311080B">https://doi.org/10.2298/VSP200311080B</a> )	Ksenija V. Bubnjević	110
Distance visual acuity in air force pilots and student pilots when exposed to + Gz acceleration in human centrifuge (DOI: <a href="https://doi.org/10.2298/VSP200607066R">https://doi.org/10.2298/VSP200607066R</a> )	Danijela Randjelović	104
Potentially inappropriate medication prescribing among elderly patients with cardiovascular diseases (DOI: <a href="https://doi.org/10.2298/VSP200623118S">https://doi.org/10.2298/VSP200623118S</a> )	Goran Stojanović	103
Influence of the health status and other relevant factors on the quality of life of elderly people (DOI: <a href="https://doi.org/10.2298/VSP210217033V">https://doi.org/10.2298/VSP210217033V</a> )	Zoran Vesić	101

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## Bacterial vaginosis – diagnostic dilemma and implications

### Bakterijska vaginoza – dijagnostičke dileme i implikacije

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#### Abstract

**Background/Aim.** Bacterial vaginosis (BV) is one of the most common microbial dysbiosis, characterized by a decrease of *Lactobacillus spp.* with an increase of other anaerobic bacteria species [*Gardnerella (G.) vaginalis*, *Atopobium (A.) vaginae*, *Prevotella spp.*, *Mobiluncus spp.*, etc.] causing serious gynecological and obstetric complications. Therefore, it is particularly important to have accurate and reliable diagnostic standards. The aim of this study was to compare the results of various diagnostic methods for detecting BV, such as Amsel, Nugent, and Ison and Hay criteria, as well as multiplex quantitative real-time polymerase chain reaction (mqRT-PCR) test. **Methods.** This study involved vaginal swabs from 235 patients of reproductive age. Nugent criteria were used as the 'gold standard' compared with Amsel and Ison/Hay criteria as well as mqRT-PCR test based on the detection and quantification of *G. vaginalis*, *A. vaginae*, *Lactobacillus spp.*, and total concentration of bacterial DNA. The  $\kappa$  coefficient was employed to measure agreement between tests. **Results.** Our analysis demonstrated excellent agreement between Ison/Hay criteria and Nugent scores ( $\kappa =$

0.95), good agreement between Amsel and Nugent criteria ( $\kappa = 0.78$ ), while between Nugent criteria and mqRT-PCR test agreement was moderate ( $\kappa = 0.59$ ). Total agreements of Ison/Hay, Amsel, and mqRT-PCR against Nugent scores were 94.9%, 90.2%, and 74%, respectively. Nugent methods classified the highest number of intermediate patients - 60 (25.2%). The largest number of BV patients was detected by the mqRT-PCR method, while the largest number of healthy patients was detected by Amsel criteria. **Conclusion.** The mqRT-PCR is the best choice for BV diagnosis because it is more efficient at differentiating patients with intermediate results. Compared to Amsel and Nugent methods that group patients into 2 or 3 categories, the mqRT-PCR method recognizes other conditions of vaginal flora important for correct diagnoses and application of better therapeutic approaches, as well as preventing possible clinical consequences of this dysbiosis.

**Key words:** diagnostic techniques, obstetrical and gynecological; microscopy; vaginal diseases; vaginal smears; vaginosis, bacterial.

#### Apstrakt

**Uvod/Cilj.** Bakterijska vaginoza (BV) je jedna od najčešćih vaginalnih disbioza, koja se karakteriše smanjenjem broja bakterija roda *Lactobacillus spp.* i povećanjem broja različitih anaerobnih bakterija [*Gardnerella (G.) vaginalis*, *Atopobium (A.) vaginae*, *Prevotella spp.*, *Mobiluncus spp.*, i dr]. S obzirom na to da BV može izazvati veliki broj ginekoloških i akušerskih komplikacija, važno je definisati tačne i pouzdane metode za njenu dijagnostiku. Cilj rada bio je da se uporede rezultati različitih dijagnostičkih metoda za detekciju BV kao što su Amsel-ovi, Nugent-ovi, Ison-ovi i Hay-ovi kriterijumi, kao i multipleks kvantitativni test *real-time polymerase chain reaction* (mkRT-PCR). **Metode.** Studijom je obuhvaćeno 235 uzoraka vaginalnih briseva pacijentkinja u reproduktivnom periodu. Kao zlatni

standard korišćeni su Nugent-ovi kriterijumi čiji su rezultati upoređeni sa Amsel-ovim, kriterijumima Ison-a i Hay-a, kao i mkRT-PCR testom, koji se bazira na detekciji i kvantifikaciji *G. vaginalis*, *A. vaginae*, *Lactobacillus spp.* i ukupnoj koncentraciji DNK prisutnih vrsta bakterija.  $\kappa$  koeficijent korišćen je kako bi se utvrdio stepen saglasnosti između korišćenih metoda. **Rezultati.** Analizom su utvrđeni sledeći stepeni saglasnosti: odličan između kriterijuma Ison/Hay-a i Nugent-a ( $\kappa = 0,95$ ), dobar između Amsel-ovih i Nugent-ovih kriterijuma ( $\kappa = 0,78$ ) i umeren između kriterijuma Nugent-a i mkRT-PCR testa ( $\kappa = 0,59$ ). Ukupan stepen saglasnosti kriterijuma Ison/Hay-a, Amsel-a i mkRT-PCR testa sa Nugent-ovom metodom je iznosio 94,9%, 90,2% i 74%, redom. Nugent-ovom metodom otkriven je najveći broj intermedijarnih nalaza

– 60 (25,2%). Najveći broj bolesnica sa BV detekovano je mkRT-PCR metodom, dok je Amsel-ovim kriterijumima utvrđen najveći broj normalnih nalaza. **Zaključak.** Najbolji izbor testa za dijagnostiku BV je mkRT-PCR, jer je efikasniji u diferenciranju bolesnica sa intermedijarnim nalazom. U poređenju sa Amsel-ovim i Nugent-ovim kriterijumima pomoću kojih se bolesnice razvrstavaju u 2 i 3 kategorije, mkRT-PCR metod prepoznaje i druga

stanja vaginalne flore, koja su važna za tačnu dijagnostiku i primenu boljeg terapijskog pristupa u lečenju BV, kao i prevenciji njenih mogućih posledica.

**Ključne reči:**  
**dijagnostičke tehnike, akušerstvo i ginekologija; mikroskopija; vagina, bolesti; vaginalni brisevi; vaginoza, bakterijska.**

## Introduction

Even though bacterial vaginosis (BV) is the most frequent and the most well-researched dysbiosis, it still presents as an enigmatic imbalance of vaginal microflora of unknown etiology<sup>1</sup>. The characteristics of BV include a reduced number of *Lactobacillus (L.) spp.* and an increase of other anaerobic bacteria<sup>2</sup>. It is identified by a lack of inflammatory symptoms and a signature metabolic pattern which includes an increased pH of the vagina (pH >4.5) and the presence of bioamine that causes a foul-smelling 'fishy' vaginal odor<sup>3,4</sup>. BV may cause serious gynecologic and obstetric complications where 50% of patients are asymptomatic<sup>5,6</sup>.

Even though the application of molecular methods in recent decades has brought many improvements in understanding the vaginal microbiome, diagnostic criteria such as the Amsel criteria originally published in the American Journal of Medicine in 1983 and Nugent criteria from 1991 have remained the 'gold standard' of clinical and research approaches to BV diagnosis. Increasingly, researchers have revealed the flaws of these standards since the application of Amsel criteria divides patients into only one of two groups (normal and BV), while Nugent criteria introduce a third (intermediate) group which represents the state between normal and BV<sup>7-9</sup>. The first researchers who highlighted the existence of other states of vaginal microflora were Ison and Hay, who added two more classes to Nugent's existing three groups – predominant cocci (orig. Grade IV) and cases where epithelial cells are visible without bacterial forms (orig. Grade 0)<sup>10</sup>.

A great number of bacteria may be detected not only in women with vaginal infections but also in healthy patients. Only with the application of multiplex quantitative real-time polymerase chain reaction (mqRT-PCR) test it is possible to confirm the quantitative relations between certain bacteria, which is crucial to enable us to differentiate between dysbiosis and eubiosis<sup>11</sup>. Previous research has confirmed that *Gardnerella G. vaginalis* and *Atopobium (A.) vaginae* are present in normal vaginal microflora. However, the high concentration of these bacteria is an important marker in BV diagnostics<sup>12</sup>. RT-PCR test is based on the detection and quantification of *G. vaginalis*, *A. vaginae*, *L. spp.*, and total concentration of bacterial DNA to differentiate between six groups of patients, including those with insufficient DNA copies and with vaginal flora of unspecified etiology.

Considering that BV is quite challenging to diagnose due to its complex polymicrobial nature, the aim of this

study was to compare the results obtained by various detection methods such as Amsel, Nugent, and Ison and Hay criteria as well as mqRT-PCR.

## Methods

This study included 235 patients of reproductive age with or without symptoms of vaginal infections. Recruitment and testing were undertaken from November 2018 to December 2019. Exclusion criteria included those with a recent history (< 2 weeks) of antibiotic use. Examination of patients and sample collection were performed at the Center for Gynecology and Human Reproduction, while sample testing was conducted at the Institute of Microbiology, the Military Medical Academy (MMA), Belgrade, Republic of Serbia. All participants gave their written consent to participate in this research. The research has been approved by the MMA Ethics Committee from December 25, 2018.

Four vaginal swabs (FLOQ Swabs, COPAN) were taken from each patient to enable further microbiological and clinical investigation. BV diagnoses were made based on existing microscopic and clinical criteria (Amsel, Nugent, Ison/Hay) but also using mqRT-PCR. Clinical examination identified the amount, consistency, and color of vaginal discharge. Vaginal pH was determined by indicator papers (Merck, pH from 4.0 to 7.0). Testing with 10% potassium hydroxide (KOH) was performed by adding a drop of 10% KOH to the swab taken from the vaginal side wall to identify the 'fishy' vaginal odor. Clue cells were identified using a wet mount slide. When using Amsel criteria, a positive diagnosis was made if 3 out of the 4 following criteria were confirmed: discharge in a homogenous grey/white color, a vaginal pH greater than 4.5, a positive test with 10% KOH of a 'fishy' vaginal odor, and the presence of clue cells. One of the swabs taken from the vaginal sidewall was used for the microscopic examination of a Gram-stained smear. Smears were examined by a Solaris biological microscope with a magnification of ×1,000. Samples of Gram-stained vaginal smears were evaluated and classified by Nugent and Ison/Hay criteria. Nugent criteria use a scoring system based on microscopic examination of Gram-stained swabs to confirm the presence and relation of Gram-positive rods (*L.*), Gram-negative and Gram-variable rods, and cocci (*G. vaginalis*, *Bacteroides spp.*) and curved Gram-negative rods (*Mobiluncus spp.*). The scoring system divides vaginal smears into three groups: normal flora (0-3 score), intermediate flora (4-6 score), and BV (7-10 score). Ison/Hay criteria classify swabs into five categories: Grade 0 (vaginal

smear without bacteria); Grade 1 (normal flora – dominance of Gram-positive rods); Grade 2 (intermediate flora – presence of Gram-positive and Gram-negative *coccobacilli*); Grade 3 (BV, dominance of Gram-variable rods and *coccobacilli* or curved Gram-positive rods); Grade 4 (dominance of Gram-positive cocci only).

Upon sampling, the swabs of the vaginal sidewalls used for the mqRT-PCR were stored in a transport medium and kept at -20 °C until DNA extraction (DNA-sorb-AM, AmpliSens). mqRT-PCR (AmpliSensFlorocenosis/Bacterial vaginosis-FRT) was used to detect and quantify *G. vaginalis*, *A. vaginae*, *L. spp.*, and the total number of bacteria. The ratio coefficients (RC),  $RC1 = \log(\text{Lac DNA}) - \log(\text{Gv} + \text{Av DNA})$ ,  $RC2 = \log(\text{Bac DNA}) - \log(\text{Lac DNA})$ , and  $RC3 = \log(\text{Bac DNA}) - \log(\text{Gv} + \text{Av DNA})$  were determined by the mutual relations between those bacteria. Based on coefficients, the patients were grouped into 6 categories: normal vaginal flora ( $RC1 > 1$ , *L. spp.* is the dominant flora); intermediate flora ( $0.5 \leq RC1 \leq 1$ , the same number of *L. spp.* and aerobic bacteria); BV ( $RC1 < 0.5$ , dominant *G. vaginalis* and *A. vaginae*); vaginal flora of non-specific etiology ( $RC2 > 1$ ,  $RC3 > 2$ , any  $RC1$  value, a small concentration of *L. spp.*, but also *G. vaginalis*, *A. vaginae*); bacterial load decrease ( $RC > 1$  but the total amount of bacteria DNA is less than  $10^6$  copies/mL and greater than  $10^5$  copies/mL); bacterial load insufficient for analysis (total amount of bacteria DNA is less than  $10^5$  copies/mL). All PCR reactions were performed on the PCR instrument (Sa-Cycler 96, Sacace, Biotechnologies).

Nugent criteria were used as the ‘gold standard’. To be able to compare the results statistically, Ison/Hay and mqRT-PCR results were divided into three groups (normal, intermediate, and BV). The Ison/Hay criteria were modified from 5 categories to 3, where Grade 0 was merged with Grade 1, and Grade 4 was merged with Grade 5. The results of the modified mqRT-PCR were divided into 3 categories. Groups

‘vaginal flora of non-specified etiology’ and ‘bacterial load insufficient for analysis’ were merged into an intermediate group. The result, ‘bacterial load decreased’, was considered a normal finding.

To enable comparison of all these criteria and because Amsel criteria have only 2 groups, all 4 methods were divided into 2 groups: non-BV and BV. Normal and intermediate results of vaginal swabs (Nugent and Ison/Hay criteria) were classified as a non-BV group. In relation to PCR criteria, all results which were non-BV (normal, intermediate, ‘bacterial load insufficient for analysis’ and ‘vaginal flora of non-specified etiology’) were grouped into one non-BV group.

The kappa coefficient was used to measure the agreement between tests. Kappa values were interpreted according to Altman<sup>13</sup>. All data were processed using the software package IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

## Results

The samples of all 235 patients were tested successfully against all four criteria for BV diagnosis. The average age of patients was 29.39 ( $\pm 6.685$ ). An overview of all results obtained through Amsel, Nugent, Ison/Hay criteria, and mqRT-PCR test are presented in Table 1. A comparison of the results between different methods (Amsel, Ison/Hay criteria, and mqRT-PCR) against the Nugent ‘gold standard’ criteria is presented in Table 2.

The measure of agreement between Amsel and Nugent criteria was reported to be good ( $kappa = 0.78$ ). The total agreement of these two criteria was 90.2%. The sensitivity of Amsel criteria in our study was 82.05%, while the specificity was 94.27%.

The agreement between the modified Ison/Hay criteria and the Nugent criteria is excellent, with a kappa coefficient of 0.95, while the total agreement is 94.9%. Ten patients with Grade 0 and Grade 4 were included in the intermediate

**Table 1**

**Overview of results using different methods for diagnosis of bacterial vaginosis (BV)**

Methods	N	INT	BV	Grade 0/ bacterial load insufficient for analysis	Grade 4	Vaginal flora of non-specified etiology	Bacterial load decreased
Amsel	162 (68.9)	-	73 (31)	-	-	-	-
Nugent	97 (41.3)	60 (25.5)	78 (33.1)	-	-	-	-
Ison/Hay	95 (40.4)	50 (21.3)	76 (32.3)	10(4.2)	4 (1.7)	-	-
mq RT-PCR	114 (48)	9 (3.8)	90 (38)	10 (4.2)	-	5 (2.1)	7 (2.9)

**N – normal flora; INT – intermediate flora; mqRT-PCR – multiplex quantitative real-time polymerase chain reaction. Results are presented as n (%)**

**Table 2**

**Comparison of different methods (Amsel, Ison/Hay criteria, and mqRT-PCR) against Nugent criteria**

	Nugent			Nugent				Nugent					
	Non-BV	BV	Total	Ison/Hay	N	INT	BV	Total	mqRT-PCR	N	INT	BV	Total
Amsel	148	14	162	N	96	9	0	105	N	90	28	3	121
Non-BV	9	64	73	INT	1	49	0	50	INT	5	14	5	24
BV	157	78	235	BV	0	2	78	80	BV	2	18	70	90
Total				Total	97	60	78	235	Total	97	60	78	235

**N – normal flora; INT – intermediate flora; BV – bacterial vaginosis; Non-BV – normal and intermediate results; mqRT-PCR – multiplex quantitative real-time polymerase chain reaction.**

under the Nugent criteria. The specificity of the Ison/Hay criteria is 100%, while the sensitivity is 98.73%. Our research showed that the biggest disagreement between these two criteria was in the intermediate group. Nine patients with intermediate results in Nugent criteria were assessed as healthy under Ison/Hay criteria, while 2 intermediate patients were classified as BV.

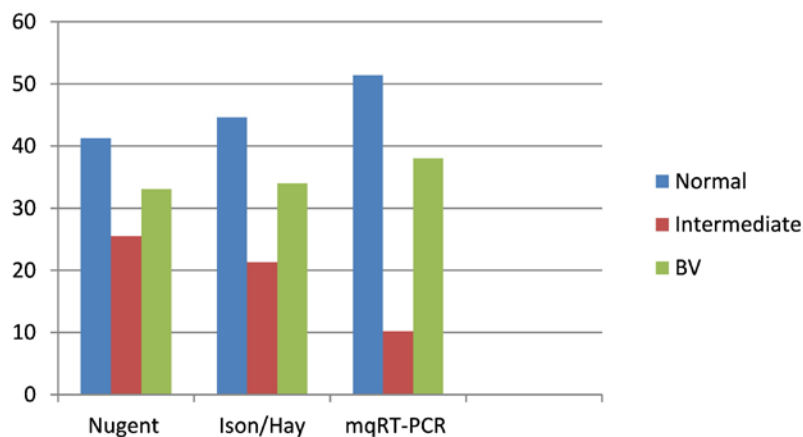
The results of the comparison of the modified mqRT-PCR test and Nugent score are shown in Table 2. The agreement between these two criteria was moderate ( $\kappa$  coefficient = 0.59), while the total agreement was 74%. The sensitivity of the PCR criteria was 89.74%, and the specificity was 87.26%. Out of the 10 patients whose bacterial load was insufficient for analysis under Nugent criteria, 6 were in the intermediate group, 2 were diagnosed with BV, and the other 2 had a normal vaginal smear. Vaginal flora of unknown etiology was detected by mqRT-PCR in 5 patients, while the Nugent criteria showed 2 patients with normal tests, 2 diagnosed with BV, and 1 with an intermediate result. Out of seven patients assessed as 'bacterial load decreased' under Nugent criteria, 2 have been classified as normal and 5 as having intermediate results.

A comparison of methods divided into three groups is shown in Figure 1. The use of Nugent criteria produced the highest number of intermediate results, 60 (25.5%), while the modified mqRT-PCR method detected only 24 (10.2%). Comparatively, the mqRT-PCR test detected the largest number of healthy patients and BV diagnoses.

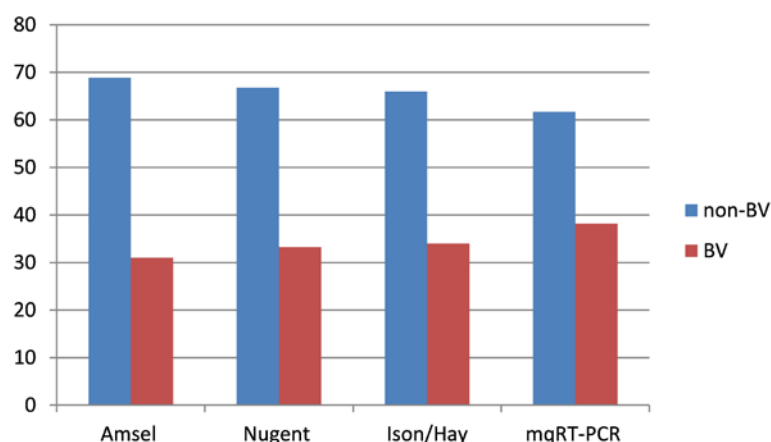
To enable the comparison of various diagnostic criteria, all four methods were divided into non-BV and BV groups (Figure 2). The highest number of BV cases was detected by the mqRT-PCR method – 90 (38.2%), while the greatest number of non-BV patients were detected by Amsel criteria – 162 (68.9%).

## Discussion

This study aimed to compare the application of different methods in the diagnosis of BV. As shown in Table 1, Amsel and Nugent criteria may define 2 and 3 groups, respectively, whereas according to Ison and Hay criteria and mqRT-PCR test, the 'other conditions' of vaginal flora have been recognized. Considering that the Nugent criteria are the most frequently used as the 'gold



**Fig. 1 – Comparison of methods divided into 3 groups (normal flora, intermediate flora, BV – bacterial vaginosis). mqRT-PCR – multiplex quantitative real-time polymerase chain reaction.**



**Fig. 2 – Comparison of criteria divided into 2 groups [nonbacterial vaginosis (non-BV) – normal and intermediate results of vaginal swabs and bacterial vaginosis (BV)]. mqRT-PCR – multiplex quantitative real-time polymerase chain reaction.**

standard' in literature, we compared other methods for BV diagnosis with the Nugent one.

Our research indicates that the 'gold standard' criterion should avoid an intermediate group. Nugent score takes into consideration only three bacterial morphotypes, which makes it difficult to comprehensively and accurately describe the diversity and complexity of the vaginal microflora. The criteria do well to describe patients with healthy or BV flora, but those with intermediate results are overlooked in most studies, remaining undefined from the point of epidemiology, clinical studies, and therapy. With the application of Nugent criteria, a significant number of women with potentially different vaginal flora (aerobic vaginitis, coinfection, mixed infection) would remain undetected. In our study, 60 (25.5%) patients were classified as intermediate by Nugent criteria which was the highest of any of the methods evaluated here.

Our results revealed the best agreement between Nugent and Ison/Hay criteria (94.9%), noting that due to our diagnostic verification, Ison/Hay criteria had been reduced to three groups. Modified Ison/Hay criteria essentially reflect Nugent classification, while the score description uses qualitative assessment for the presence of certain bacterial morphotypes instead of bacterial counts. The difference between these two methods would have been greater if we had not reduced the number of groups. Ison and Hay highlighted the fact that the microscopic results of vaginal discharge are more complex than those assessed by Nugent criteria which define only three cell morphotypes. However, the introduction of new groups is limited by the fact that the new methodology must 'survive' comparison with the gold standard, hindering the widespread implementation of these new methods.

The results of the comparison of Nugent and Ison/Hay criteria in our study are similar to the results from the original work of Ison/Hay, where the agreement of these two criteria was very good ( $kappa$  coefficient = 0.91)<sup>10</sup>. In their study, Chawla et al.<sup>14</sup> also compared these two criteria. However, their approach to statistical analysis by merging the intermediate group with normal results yielded a  $kappa$  coefficient of 0.83. Comparatively, merging the intermediate group with BV provided better agreement compared to the Nugent criteria ( $kappa$  coefficient = 0.9).

Amsel criteria are still widely employed in both research and scientific setting and are often used as the gold standard alongside Nugent criteria, according to the literature. We consider that the main flaw of this method is the classification of patients into only 2 groups despite various broad-spectrum disturbances of vaginal microflora. In our research, the agreement between these two methods is good (90.2%). Because of only two groups in Amsel criteria, Nugent criteria were reduced to BV and non-BV, which includes patients with healthy and intermediate vaginal flora. Mohammadzadeh et al.<sup>15</sup> similarly evaluated the agreement between Amsel and Nugent criteria, with results similar to our current study ( $kappa$  coefficient = 0.8). In comparison, Mahajan et al.<sup>16</sup> reported the agreement between these two criteria to be moderate, with a markedly lower efficiency score ( $kappa$  = 0.58).

Perhaps the best illustration of the flaws and problems linked to two 'gold standards' are presented in two independent studies by Sha et al.<sup>17</sup> and Amit et al.<sup>18</sup>. Based on Nugent criteria, Sha et al.<sup>17</sup> diagnosed BV in 203 patients, while only 75 were diagnosed using Amsel criteria. Therefore, 128 women were presented with false-negative results compared to the Nugent criteria. On the contrary, Amit et al.<sup>18</sup> diagnosed BV using Amsel criteria in 145 patients, while 79 were diagnosed with the BV using Nugent criteria. Therefore, 66 women were presented with false positive results. It is highly unlikely that the knowledge and skills of these two groups of researchers differ so much to obtain almost completely contradicting results. What seems like a more logical and plausible explanation is that these opposing diagnoses are the result of imperfections and flaws in current diagnostic criteria.

Accurate interpretation for Nugent scoring requires previous experience and skills in microscopy. In addition, the evaluation of the microscopic results by the Nugent method can be difficult as there are no defined criteria for differentiation of the three basic morphotypes in the scoring system. The biggest disagreements are related to issues such as what morphotypes to consider as Gram-positive rods, i.e., counting them as *L.*, what are the differences between cocci and short rods, and how to identify morphotypes bacteria such as *G. vaginalis* and *Prevotella* as they can vary in morphology from round to elongated (conditional to the coloring aspects)<sup>19</sup>. Evaluation of microscopic samples also highlights the difficulty of differentiating *G. vaginalis* and *L. iners*. *G. vaginalis* is a Gram-variable, i.e., it can be color stained as 'Gram-positive' or 'Gram-negative', while *L. iners* is difficult to identify when stained as it will present as 'Gram-negative'. That is because *L. iners* more often takes the morphological shape of *coccobacilli* than *bacilli*. The current research in microbiology indicates that these two microorganisms can be detected in healthy women as well as in those with BV (50–90%), which can lead to incorrect conclusions in categorization by Nugent score<sup>20, 21</sup>. Considering all these shortcomings, it is clear why the results of different studies and research teams differ to such a great extent.

In the last decade, with the advent of molecular methods, it has been discovered that the qualitative and quantitative diversity of the vaginal microbiome is much more complex than previously considered based on microscopic analysis and culture testing<sup>11, 22</sup>. Multiplex PCR quantitative testing used in our research detected the presence of *L. spp.*, *G. Vaginalis*, and *A. vaginae*, as well as the total DNA concentration of bacteria. Based on the mutual relationship between these bacteria, all patients were divided into 6 categories. The results showed that the largest number of BV diagnoses – 90 (38.3%), were detected using the mqRT-PCR method. The intermediate group included 15 (6.4%) patients with vaginal flora of non-specified etiology, while 9 (3.8%) patients were presented as intermediate vaginal flora.

The biggest discrepancies in our research are identified between patients classified as the intermediate group.

Twenty-eight patients classified in the intermediate group by the Nugent score were healthy, while 18 women who were assessed as intermediate by the Nugent score were diagnosed with BV in mqRT-PCR testing. Compared to the Nugent score, mqRT-PCR testing detected more patients with healthy or BV vaginal flora. Our results show moderate agreement (74%) between these two methods. However, the intermediate results obtained by the mqRT-PCR method are more reliable, which gives this method a considerable selective advantage compared to the microscopic methods. Besides, the advantage of the mqRT-PCR test is in its objectivity, the potential to quantify bacteria, and detect imbalance of flora of unknown etiology, which is important for assessing the vaginal flora status. In addition, the possibility of detecting *A. vaginae* may be important for therapeutic approaches because of the resistance of this bacteria to metronidazole. The limitation of mqRT-PCR used in this study is that it detects the presence of only two of the most frequent anaerobic bacteria, as well as an inability to distinguish species within the *L. spp* group.

Similar to our research methodology, the study done by Van den Munckhof et al.<sup>23</sup> used the same mqRT-PCR test for the detection of BV (AmpliSensFlorocenosis/Bacterial vaginosis-FRT). The findings of this study pointed out that the mqRT-PCR test showed the biggest agreement with Amsel, Nugent, culture, and BD MAX Vaginal Panel, based on 16sRNA genomic sequencing for microbiota analysis. Compared to genomic sequencing, culture sensitivity was 29.9%, Amsel method sensitivity was 61.5%, Nugent criteria and BD MAX Vaginal Panel sensitivity were 63.9%, while the mqRT-PCR test showed a sensitivity of 80.6%<sup>23</sup>. In their study, Dhiman et al.<sup>24</sup> compared the Nugent score and RT-

PCR for BV detection in 125 patients of reproductive age, and they achieved results of total agreement similar to our results (81.8%).

A limitation of the current study is our exclusion of the existence of other vaginal dysbiosis and our focus on the diagnosis of BV only. Future studies should aim to include a complete analysis of the vaginal microbiome to provide a more accurate and 'broader picture' of pathogenesis since the presence of other conditions of vaginal dysbioses, such as aerobic vaginitis and overgrowth of *Candida spp.*, can significantly influence the assessment of the vaginal microflora status.

### Conclusion

Although Nugent criteria have been used in BV diagnostics for the past 30 years, the existence of the intermediate group raises questions about the applicability of this diagnostic tool in research and scientific setting. Our results report that Nugent and Ison/Hay criteria carry the greatest accordance, followed by Amsel criteria. The mqRT-PCR method was denoted as the least in agreement with Nugent criteria, attributed to stark differences in underlying methodology and molecular biological principles. Considering that the mqRT-PCR method is more efficient at differentiating patients with intermediate results compared to Nugent and Ison/Hay methods, we concluded that mqRT-PCR is the best choice for BV diagnoses. Compared to Amsel and Nugent methods that divide patients into 2 or 3 categories, the mqRT-PCR method recognizes other conditions of vaginal flora that are important for correct diagnoses and the application of better therapeutic approaches.

### R E F E R E N C E S

1. Amabebe E, Anumba DOC. The Vaginal Microenvironment: The Physiologic Role of *Lactobacilli*. *Front Med (Lausanne)* 2018; 5: 181.
2. Van de Wijgert JHHM, Jaspers V. The global health impact of vaginal dysbiosis. *Res Microbiol* 2017; 168(9–10): 859–64.
3. Huang B, Fettweis JM, Brooks JP, Jefferson KK, Buck GA. The changing landscape of the vaginal microbiome. *Clin Lab Med* 2014; 34(4): 747–61.
4. Nelson TM, Borgogna JL, Brotman RM, Ravel J, Walk ST, Yeoman CJ. Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis?. *Front Physiol* 2015; 6: 253.
5. Gibbs RS. Asymptomatic bacterial vaginosis: is it time to treat? *Am J Obstet Gynecol* 2007; 196(6): 495–6.
6. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. *Ann Rev Microbiol* 2012; 66: 371–89.
7. Redelinghuys MJ, Geldenhuys J, Jung H, Kock MM. Bacterial Vaginosis: Current Diagnostic Avenues and Future Opportunities. *Front Cell Infect Microbiol* 2020; 10: 354.
8. Donders GGG, Ravel J, Vitali B, Netea MG, Salumets A, Unemo M. Role of Molecular Biology in Diagnosis and Characterization of Vulvo-Vaginitis in Clinical Practice. *Gynecol Obstet Invest* 2017; 82(6): 607–16.
9. Nasioudis D, Linhares IM, Ledger WJ, Witkin SS. Bacterial vaginosis: a critical analysis of current knowledge. *BJOG* 2017; 124(1): 61–9.
10. Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. *Sex Transm Infect* 2002; 78(6): 413–5.
11. Syuro LK, Fredricks DN. Microbiota of the genitourinary tract. In: Fredricks DN, editor. *The human microbiota. How microbial communities affect health and disease*. Hoboken, New Jersey: John Wiley & Sons, Inc; 2013. p. 168–99.
12. Onderdonk AB, Delaney ML, Fichorova RN. The Human Microbiome during Bacterial Vaginosis. *Clin Microbiol Rev* 2016; 29(2): 223–38.
13. Altman DG. *Practical statistics for medical research*. London: Chapman and Hall; 1991.
14. Chavla R, Bhalla P, Chadha S, Grover S, Garg S. Comparison of Hay's criteria with Nugent's scoring system for diagnosis of bacterial vaginosis. *Biomed Res Int* 2013; 2013: 365194.
15. Mohammadzadeh F, Dolatian M, Jorjani M, Alavi Majid H. Diagnostic value of Amsel's clinical criteria for diagnosis of bacterial vaginosis. *Glob J Health Sci* 2014; 7(3): 8–14.
16. Mabajan G, Mabajan A, Chopra S, Chand K. Comparison of Different Diagnostic Methods of Bacterial Vaginosis – Amsel's vs Nugent. *Int J Curr Microbiol App Sci* 2017; 6(5): 1442–8.
17. Sha BE, Chen HY, Wang QJ, Zariffard MR, Cohen MH, Spear GT. Utility of Amsel criteria, Nugent score, and quantitative PCR for Gardnerella vaginalis, Mycoplasma hominis, and Lactobacillus spp. for diagnosis of bacterial vaginosis in human immu-

- odeficiency virus-infected women. *J Clin Microbiol* 2005; 43(9): 4607–12.
18. *Amit AR, Parmjit S, Sharma V.* Comparison of the Amsel's composite clinical criteria and Nugent's criteria for diagnosis of BV: A step towards preventing mis-diagnosis. *J Adv Res Biol Sci* 2013; 5(1): 37–44.
  19. *Verstraelen H, Verhelst R.* Bacterial vaginosis: an update on diagnosis and treatment. *Expert Rev Anti Infect Ther* 2009; 7(9): 1109–24.
  20. *Forsum U, Larsson PG, Spiegel C.* Scoring vaginal fluid smears for diagnosis of bacterial vaginosis: need for quality specifications. *APMIS* 2008; 116(2): 156–9.
  21. *Verhelst R, Verstraelen P, Cools P, Lopes dos Santos Santiago G., Temmerman M, Venechoutte M.* Gardnerella. In: *Liu D*, editor. *Molecular detection of human bacterial pathogens*. Boca Raton: Press Taylor & Francis Group; 2011; p. 81–95.
  22. *Diop K, Dufour JC, Levasseur A, Fenollar F.* Exhaustive repertoire of human vaginal microbiota. *Human Microbiome J* 2019; 11: 100051.
  23. *van den Munckhof EHA, van Sitter RL, Boers KE, Lamont RF, Te Witt R, le Cessie S, et al.* Comparison of Amsel criteria, Nugent score, culture and two CE-IVD marked quantitative real-time PCRs with microbiota analysis for the diagnosis of bacterial vaginosis. *Eur J Clin Microbiol Infect Dis* 2019; 38(5): 959–66.
  24. *Dhiman N, Yoursbaw CJ, Chintalapudi MR, Turner C, Murphy E.* Diagnostic Evaluation of a Multiplex Quantitative Real-Time PCR Assay for Bacterial Vaginosis. *J Women's Health Care* 2016; 5: 1–3.

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## The impact of the complete atrioventricular block on in-hospital and long-term mortality in patients treated with primary percutaneous coronary intervention

Uticaj kompletnog atrioventrikularnog bloka na intrahospitalni i dugoročni mortalitet bolesnika lečenih primarnom perkutanom koronarnom intervencijom

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### Abstract

**Background/Aim.** The prognostic impact of complete atrioventricular (AV) block on the long-term prognosis of patients with ST-elevation myocardial infarction (STEMI) has not been fully determined. The aim of the study was to analyze the incidence and prognostic impact of complete AV block on in-hospital mortality (IHM) and 6-year mortality in STEMI patients treated with primary percutaneous coronary intervention. **Methods.** The study included 3,044 consecutive STEMI patients. **Results.** Complete AV block was registered only on admission in 144 (4.73%) patients; 125 (86.8%) patients with complete AV block had inferior infarction. A temporary pacemaker was implanted in 72 (50%) patients with complete AV block. No patient underwent permanent pacemaker implantation. IHM was significantly higher in patients with complete AV block than in patients without complete AV block: 17.9% vs. 3.6%, respectively,  $p < 0.001$ . In patients with heart block and inferior infarction, IHM was 13%, whereas IHM was 53% in patients with heart block and anterior infarction. When we

analyzed patients discharged alive from the hospital, we also found a significantly higher long-term (6-year) mortality rate in those with complete AV block vs. patients without AV block: 7.8% vs. 3.4%, respectively,  $p < 0.001$ . Complete AV block was an independent predictor for IHM and 6-year mortality: IHM [odds ratio (OR) 2.94 95%, confidence interval (CI) 1.23–5.22; 6-year mortality hazard ratio (HR) 1.61, 95%, CI 1.10–2.37]. When subanalysis was performed in patients with inferior STEMI, complete AV block was an independent predictor of IHM and 6-year mortality, while in patients with anterior STEMI, complete AV block was an independent predictor of IHM. **Conclusion.** In analyzed STEMI patients, complete AV block was transitory and was registered only on hospital admission. Although transitory, complete AV block remained a strong independent predictor of IHM and long-term mortality.

### Key words:

atrioventricular block; mortality; percutaneous coronary intervention; prognosis; st elevation myocardial infarction.

### Apstrakt

**Uvod/Cilj.** Uticaj kompletnog atrioventrikularnog (AV) bloka na dugoročnu prognozu bolesnika sa infarktomiokarda sa elevacijom ST segmenta (*ST-elevation myocardial infarction* – STEMI) nije utvrđen u potpunosti. Cilj rada bio je da se analiziraju incidenca i prognostički uticaj AV bloka na intrahospitalni mortalitet (IHM) i 6-godišnji mortalitet bolesnika sa STEMI, lečenih primarnom perkutanom koronarnom intervencijom. **Metode.** Studijom su bila obuhvaćena 3 044 konsektivna bolesnika sa STEMI. **Rezultat.** Kompletni AV blok registrovan je kod 144 (4,73%) bolesnika samo pri prijemu u bolnicu; 125

(86,8%) bolesnika sa kompletnim AV blokom imalo je infarkt donjeg zida. Privremeni pejsmejker ugrađen je kod 72 (50%) bolesnika sa kompletnim AV blokom. Stalni pejsmejker nije ugrađen ni jednom bolesniku. Kod bolesnika sa kompletnim AV blokom, IHM je bio značajno viši u poređenju sa IHM bolesnika bez kompletnog AV bloka: 17,9% vs. 3,6%,  $p < 0,001$ . Kod bolesnika sa infarktomiokardom donjeg zida i kompletnim AV blokom IHM je iznosio 13%, dok je kod bolesnika sa infarktomiokardom prednjeg zida i kompletnim AV blokom IHM iznosio 53%. Analizom rezultata bolesnika otpuštenih iz bolnice, utvrđen je značajno viši dugoročni (6-godšnji) mortalitet kod bolesnika sa kompletnim AV blokom u

poređenju sa bolesnicima bez AV bloka: 7,8% vs. 3,4%,  $p < 0,001$ . Kompletan AV blok bio je nezavisan prediktor IHM i dugoročnog, 6-godišnjeg mortaliteta: IHM [odds ratio (OR) 2,94 95%, confidence interval (CI) 1,23–5,22; 6-godišnji mortalitet hazard ratio (HR) 1,61, 95%, CI 1,10–2,37]. Dodatnom analizom je utvrđeno da je kod bolesnika sa infarktom donjeg zida kompletan AV blok bio nezavisan prediktor IHM i 6-godišnjeg mortaliteta, dok je kod bolesnika sa infarktom prednjeg zida kompletan AV

blok bio nezavisan prediktor samo IHM. **Zaključak.** Kod analiziranih bolesnika sa STEMI, kompletan AV blok bio je prolazan i registrovan je samo pri prijemu u bolnicu. Iako tranzitoran, kompletni AV blok bio je snažan nezavisni prediktor IHM i dugoročnog mortaliteta.

#### **Ključne reči:**

**srce, blok; mortalitet; perkutana koronarna intervencija; prognoza; infarkt miokarda sa st elevacijom.**

## **Introduction**

The incidence of high-degree atrioventricular (AV) block (HAVB) in patients with ST-elevation myocardial infarction (MI) – STEMI is 3–14%. Complete AV block is the most common and severe conduction disorder in these patients<sup>1</sup>. Complete AV block is registered two to four times more frequently in patients with STEMI of the inferior wall than in patients with different STEMI localization. According to data found in the literature, around 28% of inferior wall STEMIs are complicated by complete AV blocks<sup>2–6</sup>. It is well known that complete AV block complicating STEMI is associated with less favorable in-hospital outcomes, regardless of the location of the MI<sup>1, 2, 5, 7, 8</sup>. The prognostic impact of complete AV block on the long-term prognosis of patients with STEMI has not been determined with certainty, and individual authors state that it bears a greater significance in patients with anterior wall infarction than in patients with infarction localized elsewhere<sup>1, 3</sup>. Bearing in mind that the introduction of primary percutaneous coronary intervention (pPCI), as well as its perfecting, has improved the prognosis of patients with STEMI compared to thrombolysis treatment<sup>9</sup>, it is important that the authors of this paper analyze whether this contemporary therapeutic approach has influenced the incidence and the prognostic impact of complete AV block in STEMI patients in short-term and long-term follow-up<sup>1, 2, 3, 10</sup>.

The aim of this study was to analyze the incidence and prognostic impact of complete AV block on in-hospital and 6-year all-cause mortality in STEMI patients treated with pPCI.

## **Methods**

### *Study population, data collection, and definitions*

The present study enrolled 3,044 consecutive patients hospitalized between February 2006 and December 2012. The patients were included in the prospective STEMI Register at the University Clinical Center of Serbia. The purpose and objective of the STEMI Register have been previously published<sup>11, 12</sup>. The study protocol was approved by the local research Ethics Committee (No 470/II-4, from February 21, 2008). All consecutive patients with STEMI aged 18 or above, admitted to the Coronary Care Unit after undergoing pPCI in the Center, were included in the Register. Coronary angiography was performed via the femoral approach. More

detail about primary PCI and stenting of the infarct-related artery (IRA), the therapy administered to all eligible patients before pPCI (aspirin, 300 mg, and clopidogrel, 600 mg) and to the ones with visible intracoronary thrombi (GP IIb/IIIa receptor inhibitor during pPCI), has been available in previously published study<sup>12</sup>. Flow grades were assessed according to Thrombolysis in Myocardial infarction (TIMI) criteria. After pPCI, patients were treated according to current guidelines<sup>11, 12</sup>. A temporary transvenous pacemaker was implanted during pPCI if hemodynamic instability (hypotension or shock) and bradycardia with a low escape rhythm (heart rate  $< 40$  beats/min) were present.

According to the presence of a complete AV block, patients were divided into two groups: patients with complete AV block and patients without complete AV block. Demographic, baseline clinical, angiographic, and procedural data were collected and analyzed. An echocardiographic examination was performed within the first 3 days after pPCI. The central measure of left ventricular systolic function – left ventricle ejection fraction (LVEF) was assessed according to the biplane Simpson method in classical two- and four-chamber apical projections. In 10% of patients, the data about LVEF was missing. The missing data were imputed via the single imputation method. Kidney function was assessed by estimating creatinine clearance (CrCl) on admission using the Cockcroft-Gault formula.

Patients were followed up for 6 years after enrolment. Follow-up data were obtained by scheduled telephone interviews and outpatient visits.

### *Statistical analysis*

Categorical variables were expressed as frequency and percentage, while the continuous variables were expressed as the median (med) with the 25<sup>th</sup> and 75<sup>th</sup> quartiles (IQR). Analysis for the normality of data was performed using the Kolmogorov-Smirnov test. Baseline differences between groups were analyzed using the Mann-Whitney test for continuous variables and Pearson's chi-squared test for categorical variables. The Kaplan-Meier method was used for constructing the probability curves for 6-year survival, while the difference between patients, with and without complete AV block, was tested with the log-rank test. A 30-day landmark analysis was also performed. Multiple logistic regression was used to define independent predictors of in-hospital mortality (IHM) (backward method, with  $p < 0.10$  for entrance into the model). Multiple Cox analysis (backward method, with  $p <$

0.10 for entrance into the model) was used for identifying independent risk factors for the occurrence of 6-year all-cause mortality. The value of  $p < 0.05$  was considered significant. The SPSS statistical software, version 19, was applied (SPSS Inc, Chicago, IL).

## Results

Complete AV block was registered in 144 (4.73%) patients on admission; among patients with complete AV block, 125 (86.80%) had inferior infarction. No patient developed a complete post-procedural (post pPCI) AV block. Demographic, baseline clinical, laboratory, and angiographic characteristics, as well as ejection fraction (EF) in patients with and without complete AV block, are shown in Table 1.

As compared to patients without complete AV block, patients with complete AV block were older and, to a greater percentage, female. A higher percentage of these patients had cardiac insufficiency, lower systolic pressure, and lower values of CrCl on admission. Moreover, patients with complete AV block had a higher incidence of multivessel coronary disease (MVD) and a higher incidence of pre-procedural occlusion of the IRA (TIMI flow grade 0). Additionally, the value of post-procedural TIMI flow grade in the IRA in these patients was more frequently below 3 than in patients without complete AV block. Patients with complete AV block had a lower value of EF and, on average, a higher maximum value of creatine kinase. There was no significant difference in the duration of chest pain amongst the analyzed patients, but syncope prior to first medical contact was more frequent-

ly registered in patients with complete AV block. A temporary pacemaker was implanted in 72 (50%) patients with complete AV block. None of the patients underwent permanent pacemaker implantation.

IHM was significantly higher in patients with complete AV block than in patients without complete AV block: 17.9% vs. 3.6%, respectively,  $p < 0.001$ . In patients with complete AV block and inferior infarction, IHM was 13%, whereas, in patients with complete AV block and anterior infarction, IHM was 53%,  $p < 0.001$ . When 30-day landmark analysis was performed, a significantly higher 6-year mortality rate in patients with complete AV block vs. patients without complete AV block was found: 7.8% vs. 3.4%, respectively,  $p < 0.001$ . There was no difference in 6-year mortality between patients with complete AV block and inferior vs. anterior infarction: 10.3% vs. 15.32%,  $p = 0.210$ . In-hospital and long-term mortality in patients with and without complete AV block is shown in Figure 1.

The causes of death after hospital discharge in all patients with complete AV block were heart-related, e.g., sudden death, reinfarction, or worsening of heart failure.

Complete AV block was an independent predictor for in-hospital and 6-year mortality in the analyzed patients, as shown in Tables 2 and 3.

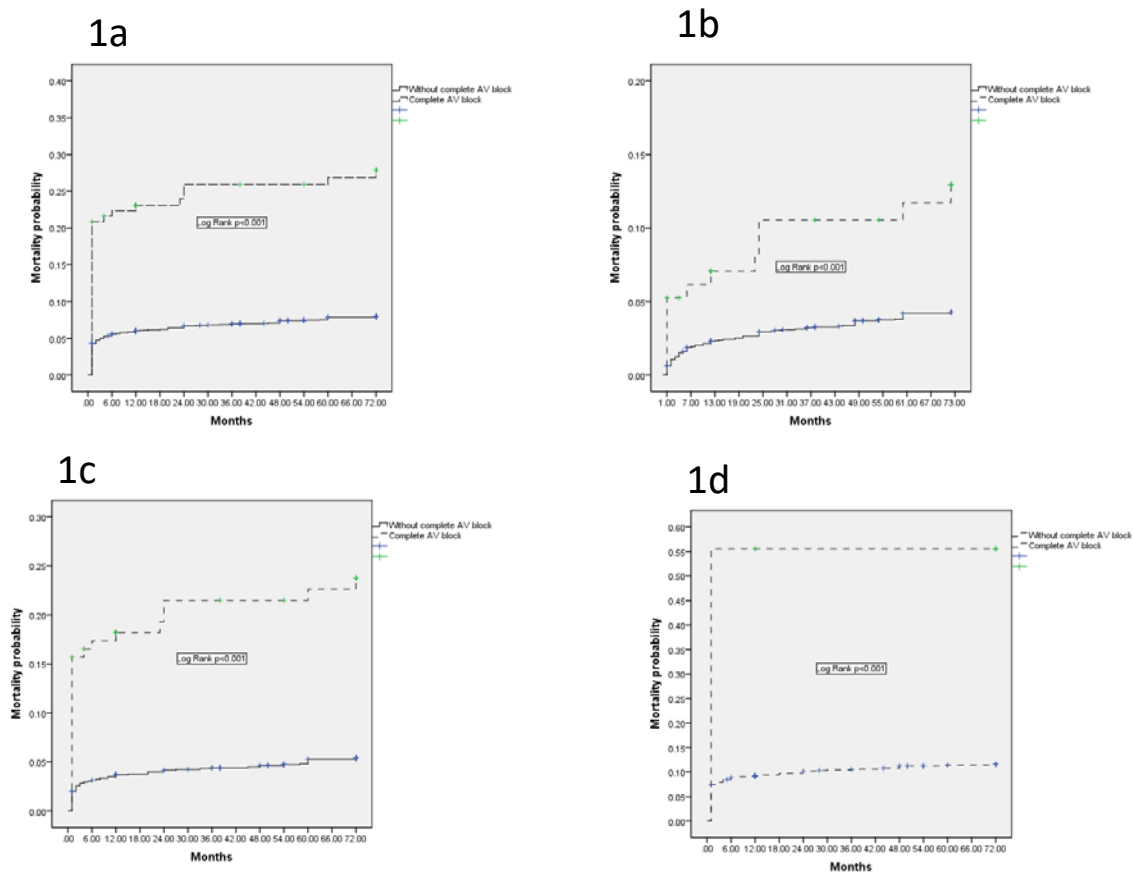
When subanalysis related to the localization of the infarction was carried out, it was found that complete AV block was an independent predictor of in-hospital [hazard ratio (HR) 2.51, 95% confidence interval (CI) 1.41–4.81,  $p = 0.001$ ] and 6-year (HR 2.52, 95% CI 1.43–3.89,  $p < 0.001$ ) mortality in patients with inferior STEMI, while in patients

**Table 1**

**Demographic, baseline clinical, laboratory, angiographic, procedural characteristics and left ventricular ejection fraction of the study patients according to the presence of complete atrioventricular (AV) block on admission**

Characteristics	Complete AV block n=144	No complete AV block n=2,900	<i>p</i> -value
Age, years, med (IQR)	64.5 (59.2,75)	60 (51,69)	< 0.001
Female, n (%)	55 (38.2)	805 (27.75)	0.011
Previous MI, n (%)	18 (12.5)	309 (10.65)	0.122
Diabetes mellitus, n (%)	35 (24.31)	575 (19.83)	0.144
Hypertension, n (%)	99 (68.75)	1999 (68.93)	0.888
HLP, n (%)	67 (46.53)	1823 (62.86)	< 0.001
Smoking, n (%)	75 (52.08)	1581 (54.51)	0.145
Pain duration, hrs, med (IQR)	3 (1,41)	3 (2,4.5)	0.658
Syncope, n (%)	8 (5.55)	37 (1.27)	< 0.001
Killip II and III on admission, n (%)	37 (88.92)	354 (12.21)	< 0.001
Killip IV on admission, n (%)	10 (6.91)	56 (1.93)	< 0.001
Systolic BP (mmHg) on admission, med (IQR)	110 (90,130)	140 (120,150)	< 0.001
Inferior infarction, n (%)	125 (86.80)	1,283 (44.24)	< 0.001
Anterior infarction, n (%)	17 (11.80)	1,230 (42.41)	0.154
Right ventricular MI, n (%)	71 (49.30)	371 (12.79)	< 0.001
3-vessel disease, n (%)	45 (31.25)	792 (27.31)	0.225
Pre-procedural flow TIMI 0, n (%)	122 (84.72)	2,225 (76.78)	0.001
Post-procedural flow TIMI < 3, n (%)	14 (9.72)	132 (4.55)	0.001
Troponin T, med (IQR)	355 (101,970)	309 (89,888)	0.971
Hemoglobin g/L, med (IQR)	136 (124,142)	142 (131,153)	< 0.001
Glucose on admission, med (IQR)	8.2 (6.6,11.2)	7 (5.9, 9.1)	< 0.001
CrCl < 60 mL/min/m <sup>2</sup> , n (%)	54 (37.5)	435 (15)	< 0.001
LVEF (%), med (IQR)	45 (35,50)	50 (40,55)	< 0.001

Med – median; IQR – interquartile range; MI – myocardial infarction; HLP – hyperlipidemia; HF – heart failure; BP – arterial blood pressure; CrCl – creatinine clearance; TIMI – thrombolysis in myocardial infarction; LVEF – left ventricle ejection fraction.



**Fig. 1 – Kaplan-Meier curves estimating the probability of 6-year mortality: a) during the entire 6-year period (in-hospital + follow-up); b) in those who survived hospitalization; c) patients with inferior myocardial infarction (MI); d) patients with anterior MI.**

AV – atrioventricular.

**Table 2**

**Independent predictors for in-hospital mortality**

Variable	OR	95% CI	p-value
Age, years	1.04	1.02–1.07	< 0.001
Post-procedural flow TIMI < 3	3.72	2.04–6.79	< 0.001
Killip class > 1 on admission	3.04	1.83–5.05	< 0.001
Complete AV block	2.94	1.23–5.22	0.011
CrCl < 60 mL/min on admission	1.96	1.09–3.53	0.024
Previous infarction	1.72	1.02–3.19	0.056
LVEF (%)	0.84	0.81–0.86	< 0.001

**TIMI – thrombolysis in myocardial infarction; AV – atrioventricular; CrCl – creatinine clearance; LVEF – left ventricle ejection fraction; OR – odds ratio; CI – confidence interval.**

**Table 3**

**Independent predictors for 6-year mortality**

Variable	HR	95% CI	p-value
Age, years	1.04	1.03–1.06	< 0.001
Post-procedural flow TIMI < 3	1.99	1.40–2.81	< 0.001
Killip class > 1 on admission	1.82	1.33–2.49	< 0.001
Complete AV block	1.61	1.10–2.37	0.017
CrCl < 60 mL/min on admission	1.45	1.04–2.02	0.028
LVEF (%)	0.92	0.90–0.93	< 0.001

**TIMI – thrombolysis in myocardial infarction; AV – atrioventricular; CrCl – creatinine clearance; LVEF – left ventricle ejection fraction; HR – hazard ratio; CI – confidence interval.**

with anterior STEMI, complete AV block was an independent predictor of only IHM (HR 4.43, 95% CI 1.05–13.77,  $p < 0.001$ ), but not of 6-year mortality.

## Discussion

The results of the present study have shown that transient complete AV block on hospital admission was present in 4.73% of analyzed patients with STEMI. Complete AV block on admission was a strong independent predictor of in-hospital and 6-year mortality in the analyzed patients. When patients were analyzed according to the localization of the infarction, complete AV block was an independent predictor of in-hospital and long-term mortality in patients with inferior STEMI, while in patients with anterior STEMI, it was an independent predictor of only in-hospital but not of long-term mortality.

The incidence of complete AV block, the clinical characteristics of the patients, and the percentage of patients with an implanted temporary pacemaker (PM) are in line with the findings obtained in the literature relating to patients treated with pPCI<sup>1, 2, 6, 13</sup>. Complete AV block complicating STEMI in the pPCI era is most commonly registered on admission as a part of acute ischemia. The most common mechanism of the occurrence of complete AV block in patients with MI is AV nodal ischemia. The nodal artery most commonly stems from the right coronary artery (RCA), which explains why complete AV block complicates inferior STEMI much more frequently than anterior STEMI. However, the AV node also receives blood from the collateral vessels and the septal branches stemming from the left anterior descending coronary artery (LAD), which is why patients with complete AV block more commonly suffer from MVD compared to patients without complete (or high degree) AV block<sup>1</sup>, which was also the case with the patients in the present study. In RCA occlusion, ischemia is most commonly above the His bundle, resulting in a satisfactory escape rhythm, which is why, in most cases, these patients do not need a temporary PM. Upon opening the RCA, AV conduction in these patients usually normalizes. In patients with LAD occlusion, ischemia is usually infra-Hisian, and the escape rhythm is usually unstable, which is why implanting a temporary PM is necessary for these patients<sup>2, 3, 4, 6, 14–18</sup>. In the present study, all patients with anterior STEMI and complete AV block had a temporary pacemaker implanted. The Bezold–Jarisch reflex is a less significant mechanism in the occurrence of complete AV block and is mostly seen in patients with inferior STEMI<sup>2, 18</sup>.

A full withdrawal of complete AV block after the opening of an infarcted artery, which has been noted in the present study, can also be found in literature analyzing patients treated with pPCI<sup>14</sup>. AV node cells have a high intracellular glycogen content making them 'resistant' to ischemia, which explains the transitory character of complete AV block in patients with STEMI and the normalization of AV conduction upon timely opening of the IRA<sup>1, 18</sup>. However, there are also studies showing that complete AV block did not withdraw in all patients after opening the IRA. In a study by Gang et al.<sup>2</sup>,

which analyzes the occurrence of HAVB complicating STEMI in the pPCI era, 9% of patients developed HAVB 48 hrs upon admission to the hospital, while persistent AV conduction disorder remained in 9% of patients with HAVB, and they had a permanent PM implanted. Furthermore, in a study by Gómez-Talavera et al.<sup>10</sup>, 3.9% of the patients had persistent complete AV block upon pPCI.

The results of the present study have shown that complete AV block remains an independent predictor of mortality even after the treatment with (contemporary) pPCI. When subanalysis in relation to the localization of the infarction was performed in the subgroup of patients with inferior MI, complete AV block remained an independent predictor of short-term and 6-year mortality. In the subgroup of patients with anterior MI, complete AV block was not an independent predictor of 6-year mortality. That can be explained by the small number of patients with anterior MI complicated by AV block released from the hospital alive. However, patients with anterior MI and complete AV block have a significantly higher 6-year mortality compared to patients with anterior MI who did not have complete AV block. It is a common finding in literature for complete AV block to be a predictor of IHM, while its effect on long-term mortality depends on the localization of the infarction and/or the patient population being analyzed. In a study by Aguiar Rosa et al.<sup>1</sup>, complete AV block was an independent predictor only of in-hospital but not of 1-year mortality in patients with MI. In this study, just like in the present study, a significantly higher IHM was registered in patients with anterior STEMI compared to patients with inferior STEMI. IHM in this study was four times higher in patients with complete AV block, while in the present study, it was 2.54 times higher. Opposed to the present study, the one by Aguiar Rosa et al.<sup>1</sup> analyzed STEMI and non-STEMI patients. Amongst the patients with STEMI, there were those treated by thrombolysis, which can explain the higher risk of IHM. On the other hand, in a study by Shacham et al.<sup>15</sup>, complete AV block complicating STEMI treated with pPCI was an independent predictor of 30-day and 5-year mortality. In this study, there were no patients with complete AV block with anterior STEMI. In a study by Kim et al.<sup>3</sup>, it was found that complete AV block was an independent predictor of long-term mortality only in patients with anterior STEMI but not in patients with inferior STEMI. The same finding can be seen in the study by Kawamura et al.<sup>4</sup>. In a study by Gang et al.<sup>2</sup>, it was found that overall long-term mortality was significantly higher in patients with HAVB compared to patients without HAVB. However, when landmark analysis was performed and patients deceased within the first 30 days were excluded, mortality between the two analyzed groups did not display significant differences. In the present study, the highest mortality was registered in the first month. However, after the 30-day landmark analysis, long-term mortality was significantly higher in patients with complete AV block compared to patients without AV block. In a study by Kosmidou et al.<sup>13</sup>, after a 30-day landmark analysis, mortality was higher in patients with HAVB compared to patients without HAVB. However, the difference disappeared after a year, which is

why there was no difference in the mortality between the analyzed groups between year one and year three of the follow-up. Although most studies indicate that, in the pPCI era, complete AV block predominantly affects intrahospital mortality in patients with STEMI, in a study by Chera et al.<sup>6</sup>, complete AV block was not found to be an independent predictor of IHM. A similar finding can be seen in a study by Lee et al., where the prognostic impact of complete AV block in patients with STEMI was analyzed, and it was not found that complete AV block influenced IHM<sup>14</sup>. The findings of the two previously cited studies may be explained by the fact that the studies were performed on a small number of patients. In addition, there is a smaller number of papers showing that complete AV does not affect mortality. In a study by Auffret et al.<sup>9</sup>, HAVB was not an independent predictor of either short-term or long-term mortality. However, the authors of that study believe that a complete AV block in their patients was a surrogate marker for a more massive infarction. The results of a study by Kim et al.<sup>19</sup>, also showed that complete AV block was not an independent predictor of 30-day adverse cardiovascular events in patients with MI. In fact, the presence of LAD as the infarcted artery was an independent predictor for the occurrence of 30-day major adverse cardiovascular events in patients with complete AV block. In the patients from the present study, complete AV block remained an independent predictor of mortality, along with cardiac insufficiency and lower EF of the left ventricle, as “stronger” markers indicating extensive necrosis of the myocardium, i.e., a more massive infarction.

The data shown in our study represent an addition to the existing knowledge on the unfavorable prognostic influence of complete AV block in patients with STEMI. At this moment, there is no ideal therapeutic strategy that would improve the prognosis of patients with STEMI and complete AV block. In the current absence of new knowledge and therapy options, patients with STEMI and complete AV block require special attention during hospitalization, more frequent check-ups after hospital discharge, and more “aggressive” secondary prevention of coronary artery disease.

### Study limitations

The limitations of the present study need to be acknowledged. The study is observational, but it is controlled, prospective, and has included consecutive patients, thus limiting possible selection bias. In the present study, patients were treated with clopidogrel. No patients were treated with more recently developed antiplatelet drugs (prasugrel and/or ticagrelor), and pPCI was predominantly performed using bare metal stents. Ticagrelor, prasugrel, and/or the new generation of drug-eluting stents or biodegradable polymers were not available for routine administration to patients at the time of their enrollment into the Register, which may have influenced the prognosis of the analyzed patients. The study was not designed to evaluate whether changing any treatment strategy would impact the short- and long-term outcomes of the patients analyzed.

### Conclusion

In STEMI patients treated with contemporary pPCI, complete AV block was transitory and was registered only on hospital admission. In-hospital and 6-year mortality was significantly higher in patients with complete AV block compared to patients without complete AV block. Although transitory, complete AV block was a strong independent predictor of in-hospital and long-term mortality in the analyzed patients.

### Disclosure statement

The authors report no financial relationships or conflicts of interest regarding the content herein.

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## R E F E R E N C E S

1. Aguiar Rosa S, Timóteo AT, Ferreira L, Carvalho R, Oliveira M, Cunha P, et al. Complete atrioventricular block in acute coronary syndrome: prevalence, characterisation and implication on outcome. *Eur Heart J Acute Cardiovasc Care* 2018; 7(3): 218–23.
2. Gang UJ, Hvelplund A, Pedersen S, Iversen A, Jons C, Abildstrom SZ, et al. High-degree atrioventricular block complicating ST-segment elevation myocardial infarction in the era of primary percutaneous coronary intervention. *Europace* 2012; 14(11): 1639–45.
3. Kim KH, Jeong MH, Ahn Y, Kim YJ, Cho MC, Kim W, et al. Differential Clinical Implications of High-degree Atrioventricular Block Complicating ST-Segment Elevation Myocardial infarction according to the Location of Infarction in the Era of Primary Percutaneous Coronary Intervention. *Korean Circ J* 2016; 46(3): 315–23.
4. Kawamura Y, Yokoyama H, Kitayama K, Miura N, Hamadate M, Nagawa D, et al. Clinical impact of complete atrioventricular block in patients with ST-segment elevation myocardial infarction. *Clin Cardiol* 2021; 44(1): 91–9.
5. Yadav S, Yadav H, Dwivedi SK, Parasbar NK, Chandra S, Chaudhary G, et al. The time to reversal of complete atrioventricular block and its predictors in acute ST-segment elevation myocardial infarction. *J Electrocardiol* 2020; 63: 129–33.
6. Chera HH, Mitre CA, Nealis J, Mironov A, Budzikowski AS. Frequency of Complete Atrioventricular Block Complicating ST-Elevation Myocardial infarction in Patients Undergoing Primary Percutaneous Coronary Intervention. *Cardiology* 2018; 140(3): 146–51.
7. Jim MH, Chan AO, Tse HF, Barold SS, Lau CP. Clinical and angiographic findings of complete atrioventricular block in acute inferior myocardial infarction. *Ann Acad Med Singap* 2010; 39(3): 185–90.
8. Aplin M, Engstrom T, Vejstrup NG, Clemmensen P, Torp-Pedersen C, Køber L. Prognostic importance of complete atrioventricular

- block complicating acute myocardial infarction. *Am J Cardiol* 2003; 92(7): 853–6.
9. Auffret V, Loirat A, Leurent G, Martins RP, Filippi E, Conderet I, et al. High-degree atrioventricular block complicating ST segment elevation myocardial infarction in the contemporary era. *Heart* 2016; 102(1): 40–9.
  10. Gómez-Talavera S, Vivas D, Pérez-Vizcayno MJ, Hernández-Antolín R, Fernández-Ortiz A, Bañuelos C, et al. Prognostic implications of atrio-ventricular block in patients undergoing primary coronary angioplasty in the stent era. *Acute Card Care* 2014; 16(1): 1–8.
  11. Mrdović I, Savić L, Lasica R, Krljanac G, Asanin M, Brdar N, et al. Efficacy and safety of tirofiban-supported primary percutaneous coronary intervention in patients pretreated with 600 mg clopidogrel: results of propensity analysis using the Clinical Center of Serbia STEMI Register. *Eur Heart J Acute Cardiovas Care* 2014; 3(1): 56–66.
  12. Savić L, Mrdović I, Asanin M, Stanković S, Matić D, Krljanac G, et al. Impact of the combined presence of left ventricular systolic and renal dysfunction on the 5-year outcome after ST-elevation myocardial infarction. *Vojnosanit Pregl* 2015; 72(8): 702–9.
  13. Kosmidou I, Redfors B, Dordi R, Dizon JM, McAndrew T, Mebran R, et al. Incidence, Predictors, and Outcomes of High-Grade Atrioventricular Block in Patients With ST-Segment Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention (from the HORIZONS-AMI Trial). *Am J Cardiol* 2017; 119(9): 1295–301.
  14. Lee SN, Hwang YM, Kim GH, Kim JH, Yoo KD, Kim CM, et al. Primary percutaneous coronary intervention ameliorates complete atrioventricular block complicating acute inferior myocardial infarction. *Clin Interv Aging* 2014; 9: 2027–31.
  15. Shacham Y, Lesbem-Rubinow E, Steinvil A, Keren G, Roth A, Arbel Y. High Degree Atrioventricular Block Complicating Acute Myocardial Infarction Treated with Primary Percutaneous Coronary Intervention: Incidence, Predictors and Outcomes. *Isr Med Assoc J* 2015; 17(5): 298–301.
  16. Hwang YM, Kim CM, Moon KW. Periprocedural temporary pacing in primary percutaneous coronary intervention for patients with acute inferior myocardial infarction. *Clin Interv Aging* 2016; 11: 287–92.
  17. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2018; 39(2): 119–77.
  18. Singh SM, FitzGerald G, Yan AT, Brieger D, Fox KA, López-Sendón J, et al. High-grade atrioventricular block in acute coronary syndromes: insights from the Global Registry of Acute Coronary Events. *Eur Heart J* 2015; 36(16): 976–83.
  19. Kim HL, Kim SH, Seo JB, Chung WY, Zo JH, Kim MA, et al. Influence of second- and third-degree heart block on 30-day outcome following acute myocardial infarction in the drug-eluting stent era. *Am J Cardiol* 2014; 114(11): 1658–662.

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# Macular morphologic changes following successful retinal detachment repair by scleral buckling surgery

Morfološke promene makule nakon uspešne operacije ablacije retine metodom „nabiranja“ sklere

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## Abstract

**Background/Aim.** Spectral domain optical coherence tomography (SDOCT) is very useful for the accurate examination of macular microstructure. The aim of this study was to evaluate macular morphologic changes after successful retinal detachment (RD) surgery by scleral buckling (SB) using SDOCT and assess their impact on vision repair. **Methods.** SDOCT examination was performed in 1, 6, and 12 months in 27 eyes following SB surgery with the successful anatomical repair of rhegmatogenous RD, which also affected the macular region. The examination was performed in a 6 mm diameter central macular region and included measurements of the central foveal thickness, average total retinal thickness (TRT), and thickness of the inner and outer retinal layer (ORT) separately. The numerical values of parameters for each operated eye were compared with those of the fellow (control) eye of each patient. The condition of the external limiting membrane (ELM) and inner segment (IS) and outer segment (OS) of the photoreceptors was also examined. **Results.** The mean TRT and ORT in the reattached regions in the operated eyes were significantly thinner than the corresponding regions of the fellow (control) eye, and throughout the follow-up period, the difference was statistically significant. There was a statistically significant difference in TRT (after 1 month,  $p =$

0.021, after 6 months,  $p = 0.026$ , after 12 months,  $p = 0.027$ ) and ORT (after 1 month,  $p = 0.018$ , after 6 months,  $p = 0.019$ , after 12 months,  $p = 0.021$ ) between the eyes with a longer preoperative duration of macular detachment (MD) of 2 weeks and eyes with shorter detachment period. Disruptions of the IS and OS of photoreceptors and ELM on SDOCT examination after one month were observed in 37.04% of eyes, after 6 months in 29.6% of eyes, and at the end of the follow-up period in 14.8% of eyes. A statistically significant difference was found in the frequency of disruptions of the IS and OS, and ELM depending on the preoperative duration of RD ( $p = 0.007$ ). **Conclusion.** The overall decrease in the mean retinal thickness after successful anatomical repair of RD is the result of a decrease in the thickness of the outer retinal layers. The alterations of the ELM, IS and OS of photoreceptors observed on the early SDOCT scans are mostly associated with limited vision recovery. The prolonged MD leads to damaging the neurosensory tissue of the retina and especially the photoreceptors, which may explain the limited visual acuity recovery after successful SB repair of RD.

## Key words:

eye diseases; macula lutea; retinal detachment; scleral buckling; tomography, optical coherence; treatment outcome; visual acuity.

## Apstrakt

**Uvod/Cilj.** Optička koherentna tomografija spektralnog domena (OKTSD) je veoma korisna za precizno ispitivanje mikrostrukture makule. Cilj rada bio je da se procene morfološke promene makule nakon uspešne operacije ablacije retine (AR) metodom „nabiranja“ sklere koristeći metod OKTSD kao i procena njihovog uticaja na oporavak vida. **Metode.** Pregled OKTSD-om obavljen je na 27 očiju 1, 6 i 12 meseci nakon operacije „nabiranja“

sklere sa uspešnom anatomskom reparacijom regmatogene AR koja je istovremeno zahvatala i makularnu regiju. Pregled je bio izvršen u centralnom regionu makularne regije, promera 6 mm i uključivao je merenje centralne fovealne debljine, prosečne ukupne debljine retine (UDR) i debljine unutrašnjeg i spoljašnjeg sloja retine (SSR) pojedinačno. Numeričke vrednosti parametara svakog operisanog oka upoređene su sa vrednostima drugog (kontrolnog) oka istog bolesnika. Takođe, izvršena je analiza integriteta unutrašnjeg segmenta (US) i spoljašnjeg



segmenta (SS) fotoreceptora i integriteta spoljašnje granične membrane (SGM). **Rezultati.** Tokom celokupnog perioda praćenja prosečna UDR i debljina SSR u repariranim regijama operisanih očiju bile su statistički značajno manje od odgovarajućih regija kontrolnih očiju. Postojala je statistički značajna razlika u UDR (nakon 1 meseca  $p = 0,021$ , posle 6 meseci  $p = 0,026$ , posle 12 meseci  $p = 0,027$ ) i debljini SSR (posle 1 meseca  $p = 0,018$ , posle 6 meseci  $p = 0,019$ , nakon 12 meseci  $p = 0,021$ ) između očiju sa preoperativnim trajanjem ablacije makule (AM) dužim od 2 nedelje i očiju sa kraćim preoperativnim trajanjem AM. Poremećaji US i SS fotoreceptora i SGM primećeni su na OKTSD pregledu nakon prvog postoperativnog meseca kod 37,04% očiju, nakon 6 meseci kod 29,6% očiju, a na kraju perioda praćenja kod 14,8% očiju. Utvrđena je

statistički značajna razlika u učestalosti ovih promena u zavisnosti od preoperativnog trajanja AR ( $p = 0,007$ ). **Zaključak.** Sveukupno umanjeње srednje debljine retine nakon uspešne anatomske reparacije AR rezultat je smanjenja debljine njenih spoljašnjih slojeva. Promene SGM, US i SS fotoreceptora uočene na ranim OKTSD snimcima uglavnom su povezane sa ograničenim oporavkom vida. Duže trajanje AM dovodi do oštećenja neurosenzornog tkiva retine, posebno fotoreceptora, što može objasniti nepotpun oporavak oštine vida nakon uspešne reparacije AR metodom „nabiranja“ sklere.

#### **Ključne reči:**

**oko, bolesti; žuta mrlja; retina, ablacija; beonjača, kopča; tomografija, optička, koherentna; lečenje, ishod; oština vida.**

## **Introduction**

Rhegmatogenous retinal detachment (RRD) is a retinal disorder in which the neurosensory retina separates from retinal pigment epithelium (RPE). It is caused by one or more retinal breaks that allow vitreous fluid to pass through and collect in the potential space between the neurosensory retina and RPE. It causes various morphologic changes in the macular architecture and visual acuity (VA) reduction.

The scleral buckling (SB) procedure can be used successfully to treat the majority of RRD and represents a widely accepted surgical treatment for this retinal disorder. However, despite a successful anatomical repair, vision repair results do not reflect this high rate of anatomical success<sup>1, 2</sup>. When the retinal detachment (RD) involves the macula prior to surgery, the recovery of VA is often limited and usually does not return to baseline. In eyes with detached macula preoperatively, only 37% achieve 0.4 or better VA despite the anatomical success of the surgery of 90%<sup>3</sup>.

The existence of microstructural macular changes that are not visible by fundus biomicroscopy can explain limited vision recovery after anatomically successful retinal reattachment<sup>4</sup>. That can be explained by various changes such as persistent residual subretinal fluid (RSRF), development of epimacular epiretinal membrane (EEM), cystoid macular edema (CME), or retinal folds and pigment migration<sup>5-7</sup>.

Various histopathological changes occur during RD. It has been shown in experimental models that retinal tissue degeneration occurs very early following RD<sup>8-10</sup>.

The nutrition and oxygen supply of the outer retinal layers (ORL) is one of the main problems after RD development. That causes prominent retinal structural changes, especially in photoreceptors and the outer nuclear layer (ONL). In terms of poor vision recovery in RRD, various factors were assessed, such as disruption of photoreceptors of the inner segment (IS) and outer segment (OS) and external limiting membrane (ELM)<sup>11, 12</sup>.

After successful surgery, a few microscopic studies in the reattached human retina revealed the atrophy of the ORL, especially photoreceptor layers<sup>13, 14</sup>. Because these micro-

scopic changes are very discrete, they are not often clinically visible. Recent development in spectral domain optical coherence tomography (SDOCT) allows more detailed retinal layers evaluation and provides better resolution of intraretinal structures<sup>15</sup>.

In this study, we have tried to identify changes in retinal microstructure after SB surgery in successfully reattached retinas compared to fellow healthy eyes using SDOCT, as well as to estimate the influence of these changes on vision recovery. Moreover, based on SDOCT images, we quantified the thickness of all retinal layers and compared the thickness of reattached regions with the corresponding regions of the fellow healthy eye.

## **Methods**

The study was performed at the Clinic of Ophthalmology, University Clinical Center Kragujevac, Serbia. The study was conducted in a period from 2017 to 2020. That was a prospective observational follow-up study of 27 patients following successful anatomical repair of RRD, which preoperatively also affected the macular region by SB surgery.

The study group of 27 eyes has been taken from a larger group of eyes that underwent SB and included only the eyes in which complete retinal repair was achieved after the first operation during the entire postoperative follow-up period. The eyes with retinal re-detachment during the follow-up period and requiring any additional intervention, either repeated SB or *pars plana* vitrectomy (PPV), were excluded from the study.

Only patients with successfully repaired RRD with macular involvement by a single SB procedure without any intra and postoperative complications were included in this study. Only eyes with transparent ocular media were included in this study. Eyes with pre-existing ocular diseases or previous ocular surgery, except cataract surgery, were not included in the study.

The existence of various macular diseases in the operated and fellow eyes, such as diabetic retinopathy, retinal vascular occlusion, age-related macular degeneration, prolifera-

tive vitreo-retinopathy, conditions that affect the vitreo-macular interface (e.g., epiretinal membrane and macular hole), the presence of glaucoma and uveitis or high myopia exceeding -6,0 diopters, were all exclusion criteria for this study.

Full ophthalmologic examination was performed preoperatively and postoperatively, including VA measurement (using standard Snellen eye charts), measurement of intraocular pressure with Goldmann applanation tonometer, fundus examination with indirect ophthalmoscopy and fundus biomicroscopy with Goldmann three-mirror contact lens and Volk 78 lenses, which make it possible to determine the extent and grading of RD.

With the approval of the institutional Ethics Committee (No 03/17-3807, from December 18, 2017) and according to the tenets of the Declaration of Helsinki, all enrolled patients gave their written consent at the beginning of the investigation.

In all patients, in the ophthalmologic operating room, under general anesthesia, standard SB surgery was done. In short, after a 360° limbal peritomy and placement of traction sutures beneath the rectus muscles, retinal breaks were identified by indirect ophthalmoscopy. Retinal breaks were treated with trans-scleral cryotherapy to freeze the outer surface of the eye. External drainage of the subretinal fluid (SRF) was avoided whenever possible and performed only when necessary.

Mattress sutures were placed with 5-0 polyester (Assut Astralen, Pully-Lausanne, Switzerland), and segmental radial or circumferential silicone scleral explant (Geuder AG, Heidelberg, Germany) was finally positioned to close the break(s). Finally, a 3.5 mm encircling circumferential band 360° buckle (Geuder AG, Heidelberg, Germany) was used and sutured with 5-0 polyester. At the end of the surgery, Dexamethasone and Gentamicin were injected subconjunctivally. Ofloxacin 0.3% and Dexamethasone 0.1% were applied topically five times a day for two postoperative weeks.

SDOCT examination was performed at least one month after surgery and then after 6 and 12 months (Optopol REVO NX 130 SDOCT, OPTOPOL Technology, Zawiercie, Poland). The examination and measurements of retinal layer thickness were performed in a 6 mm diameter central macular region, including nine macular sub-fields according to the Early Treatment Diabetic Retinopathy Study grid cells scheme (ETDRS). The central foveal thickness (CFT) was measured, and also the measurement of average overall retinal thicknesses in another eight analyzed macular fields was made. An automated segmentation protocol was used for thickness measurements of the ORL and inner retinal layers (IRL). The outer retina encompassed the following retinal layers: photoreceptor layer (PRL), ONL, and outer plexiform layer (OPL), i.e., marked as a distance from the proximal OPL boundary to the proximal RPE boundary. The inner retina encompassed the following retinal layers: inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), and nerve fiber layer (NFL), i.e., marked as the distance from the internal limiting membrane (ILM) to the distal boundary of the INL. Furthermore, the thickness of NFL,

GCL, and IPL was measured separately. The numerical values of operated and fellow (control) eyes were compared.

The condition of the photoreceptors of the IS and OS, and ELM was examined in particular detail, and so was the existence of other retinal changes such as residual subfoveal fluid (RSFF), cystic retinal spaces, and epimacular membranes (EMM).

SDOCT scans were then independently evaluated by two experienced retinal specialists.

In analyzing statistical data, SPSS version 22 (IBM Corp., Armonk, NY, USA) was used. The statistical differences in retinal thickness between the analyzed and control eyes were assessed by using a two-tailed *t*-test. Kruskal Wallis test was used for testing the changes in VA during the follow-up period. The chi-squared test ( $\chi^2$ -test) was used to examine the incidence of the disruption of photoreceptors in the IS and OS, ELM, RSFF, CME, and EM. In the case of intergroup comparison, the Mann-Whitney U test was used. A value of *p* lower than 0.05 was considered statistically significant.

## Results

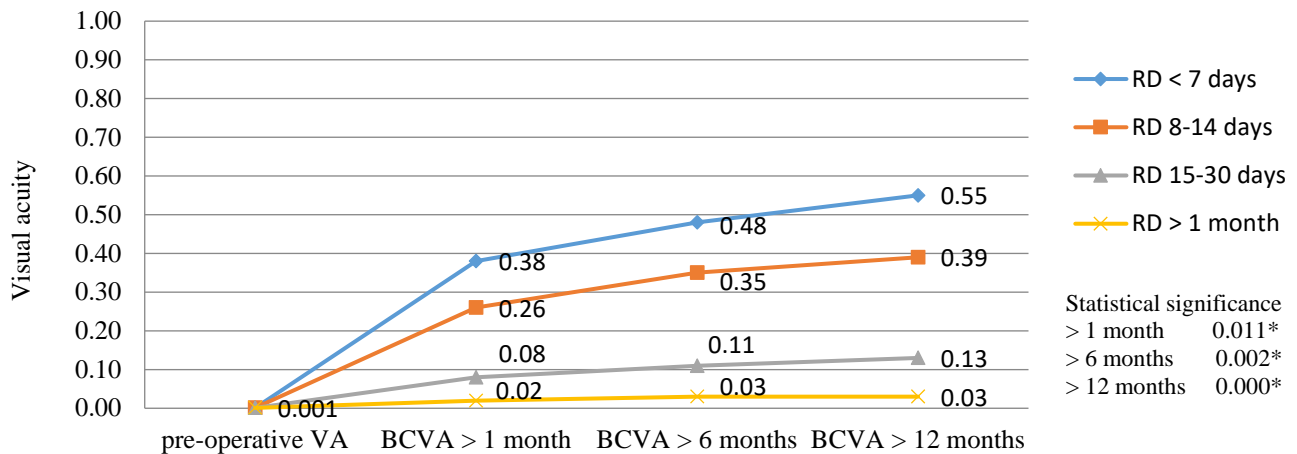
The current study included 27 eyes of 27 patients. The mean age of patients was  $51.7 \pm 12.1$  years (27–67 years). RD was noted in 16 females and 11 males, in the right eye in 17 patients, and in the left eye in 10 patients. Nineteen eyes (70.4%) were phakic, and the other 8 (29.6%) were pseudophakic. Thirteen eyes had a superior RD in one retinal quadrant, 8 had RD in 2 upper retinal quadrants, and the other 6 had RD in all four retinal quadrants. The average number of retinal breaks was  $1.6 \pm 1.1$  (1-3) and, in all cases, they were located in superior retinal quadrants.

In all eyes, the macula was detached before the surgery. The mean [ $\pm$  standard deviation (SD)] duration of macular detachment (MD) was 1 to 42 days (mean,  $13.0 \pm 11.5$  days). Eleven (40.7%) eyes had MD for less than 7 days, 8 (29.6%) eyes between 8-14 days, 5 (18.5%) eyes between 15–30 days, and another 3 (11.1%) for over 1 month.

Anatomic success (complete retinal reattachment) was noted in all cases after one surgical intervention, and the macula was successfully reattached in all cases. All surgeries went satisfactorily without any significant intra or postoperative complications. No vortex veins and muscles were damaged during the operation.

Figure 1 shows postoperative values of VA during the follow-up period, depending on the duration of RD before surgery. In all eyes, since there was macula detachment (MD), the preoperative VA was also very low and ranged from finger counting to hand motion.

In the eyes with a preoperative duration of RD for less than 7 days, the mean best corrected visual acuity (BCVA) was  $0.38 \pm 0.17$  at the end of the first month, increasing to  $0.48 \pm 0.15$  at the end of the sixth month, and to  $0.55 \pm 0.12$  at the end of the twelfth month. In the eyes with a preoperative duration of RD between 8–14 days, the mean BCVA was  $0.26 \pm 0.11$  at the end of the first month, increasing to  $0.35 \pm 0.09$  at the end of the sixth month, and to  $0.39 \pm 0.09$



**Fig. 1 – Postoperative values of best corrected visual acuity (BCVA) during the follow-up period, depending on the duration of retinal detachment (RD) before surgery.**  
 VA – visual acuity.

at the end of the twelfth month. In the eyes with a preoperative duration of RD between 15–30 days, mean BCVA was  $0.08 \pm 0.03$  at the end of the first month, increasing to  $0.11 \pm 0.05$  at the end of the sixth month, and to  $0.13 \pm 0.07$  at the end of the twelfth month. In the eyes with a preoperative duration of RD longer than 1 month, the mean BCVA was at the end of the first, sixth, and twelfth month  $0.02 \pm 0.01$ ,  $0.03 \pm 0.01$ , and  $0.03 \pm 0.01$ , respectively. During the entire follow-up period, between eyes with preoperative RD duration for less than 14 days and eyes with a longer detachment duration, there was a statistically significant difference in postoperative VA (after 1 month,  $p = 0.011$ , after 6 months,  $p = 0.002$ , after 12 months,  $p = 0.000$ ).

Table 1 shows the results of the SDOCT segmentation analysis and comparison of the thickness of the retinal layers between the reattached regions of the operated eye and corresponding regions in control (fellow) eyes.

In eyes that underwent SB, the mean CFT, total retinal thickness (TRT), outer retinal thickness (ORT), inner retinal thickness (IRT), and NFL + GCL + IPL thickness in the reattached regions were thinner than the corresponding region in the fellow eye. TRT and ORT in the reattached regions in the operated eyes were significantly thinner than the corresponding regions of the fellow eye, and throughout the follow-up period, the difference was statistically significant. Initial postoperative SDOCT after 1 month showed that the mean TRT and ORT in the reattached region were  $296.6 \pm 8.3 \mu\text{m}$  and  $140.1 \pm 4.3 \mu\text{m}$ , whereas the mean thicknesses of these layers in the corresponding regions of the fellow eyes were  $314.1 \pm 6.3 \mu\text{m}$  and  $151.8 \pm 4.3 \mu\text{m}$ , respectively. Statistical analysis of the variables showed that the difference was statistically significant compared to the value of control (fellow) eyes ( $p = 0.028$ ,  $p = 0.031$ , respectively). On the follow-up, SDOCT 6 and 12 months later, the mean ORT in the reattached region was  $141.5 \pm 7.1 \mu\text{m}$  and  $142.6 \pm 4.3 \mu\text{m}$ , respectively. The difference was statistically significant after statistical analysis of the variables compared to the fellow eyes ( $p = 0.018$ ,  $p = 0.019$ , respectively).

Table 2 shows the differences in the thickness of retinal layers of the reattached retinal area in the operated eyes depending on the preoperative detachment duration.

In operated eyes with a longer preoperative duration of RD, the mean CFT, TRT, ORT, IRT, and NFL + GCL + IPL thickness values were lower than in eyes with shorter detachment duration. By intergroup comparison, during the entire follow-up period, there was a statistically significant difference in TRT depending on the preoperative detachment duration (after 1 month,  $p = 0.021$ , after 6 months,  $p = 0.026$ , after 12 months,  $p = 0.027$ ). In addition, a statistically significant difference was present in ORT (after 1 month,  $p = 0.018$ , after 6 months,  $p = 0.019$ , after 12 months,  $p = 0.021$ ).

On SDOCT examination, disruptions and abnormalities of ELM and the photoreceptors in IS and OS were noticed in 10 eyes (37.04%) after one month, in 8 eyes (29.6%) after 6 months, and in 4 eyes (14.8%) at the end of the follow-up period after 12 months. In eyes with preoperative RD shorter than 7 days, these abnormalities were observed on SDOCT examination after 1 and 6 months in only 1 (9.1%) eye, but these changes were not detected in any eyes in this group at the end of the follow-up period. In the eyes with preoperative RD between 8–14 days, these abnormalities were observed on SDOCT examination after 1 month in 3 (37.5%) eyes, after 6 months in 2 (25.0%) eyes, and after 12 months, these abnormalities were not detected in any eyes in this group. In eyes with preoperative RD between 15–30 days, these abnormalities were detected in 3 (60.0%) eyes after the first month, after 6 months in 2 (40.0%) eyes, and after 12 months in only 1 (20.0%) eye. In eyes with preoperative RD longer than one month, disruptions of IS and OS, and ELM existed in all 3 (100%) eyes during all SDOCT recordings for the entire follow-up period.

There was a statistically significant difference in the frequency of disruptions of the photoreceptor IS and OS, and ELM depending on the preoperative duration of RD between eyes with a preoperative RD duration shorter than 2 weeks

**Table 1** Comparison of the thickness of retinal layers of the reattached regions in the operated eyes between corresponding regions in the fellow eyes

OCT	CFT (µm)		TRT (µm)		ORT (µm)		IRT (µm)		NFL+GCL+IPL thickness (µm)	
	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	n
> 1 month	205.9 ± 7.7	27	296.6 ± 8.3	27	140.1 ± 4.3	27	157.2 ± 7.5	27	118.1 ± 6.2	27
> 6 months	207.8 ± 8.1	27	299.0 ± 9.2	27	141.5 ± 7.1	27	157.4 ± 8.4	27	118.7 ± 7.1	27
> 12 months	209.5 ± 8.9	27	300.1 ± 9.8	27	142.6 ± 4.3	27	157.7 ± 9.3	27	118.8 ± 7.6	27

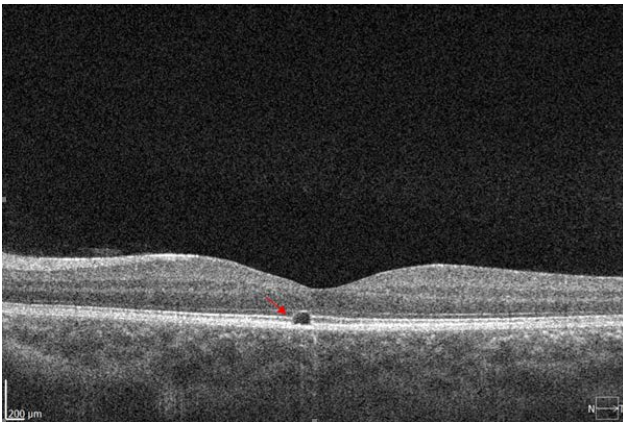
OCT – optical coherence tomography; F – fellow (control) eyes; O – operated eyes; CFT – central foveal thickness; TRT – total retinal thickness; ORT – outer retinal thickness; IRT – inner retinal thickness; NFL – nerve fiber layer; GCL – ganglion cell layer; IPL – inner plexiform layer; SD – standard deviation.

**Table 2** Differences in thickness of retinal layers of the reattached regions in the operated eyes depending on the duration of retinal detachment throughout the follow-up period

Duration of RD (days)	CFT (µm)		TRT (µm)		ORT (µm)		IRT (µm)		NFL+GCL+IPL thickness (µm)	
	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	n
< 7 (n = 11)	207.1 ± 5.8	11	301.8 ± 3.1	11	146.3 ± 3.6	11	158.8 ± 5.7	11	121.0 ± 4.3	11
8–14 (n = 8)	203.2 ± 4.2	8	298.5 ± 6.5	8	142.6 ± 3.8	8	158.0 ± 6.4	8	118.9 ± 4.3	8
15–30 (n = 5)	194.7 ± 3.9	5	293.1 ± 6.2	5	133.4 ± 3.9	5	158.1 ± 9.3	5	119.6 ± 2.6	5
> 30 (n = 3)	193.3 ± 6.3	3	281.3 ± 8.3	3	130.0 ± 1.2	3	156.3 ± 7.4	3	118.2 ± 5.7	3
<i>p</i>	0.051		0.026		0.019		0.079		0.092	

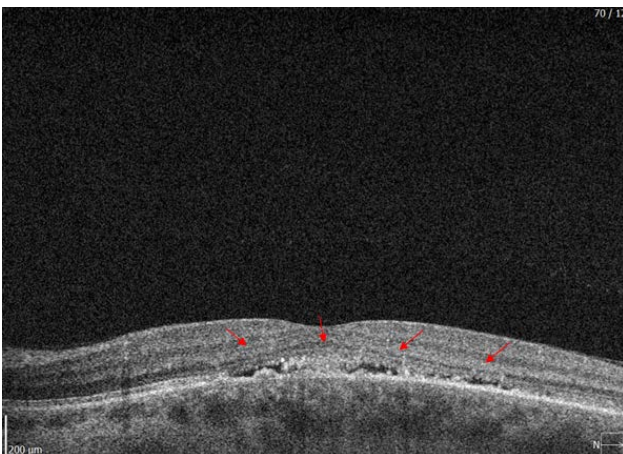
RD – retinal detachment; CFT – central foveal thickness; TRT – total retinal thickness; ORT – outer retinal thickness; IRT – inner retinal thickness; NFL – nerve fiber layer; GCL – ganglion cell layer; IPL – inner plexiform layer; m – month(s).

and eyes with a longer RD duration ( $p = 0.007$ ). Figure 2 shows subtle IS and OS photoreceptors and ELM disruptions in the eye with a short duration of MD.



**Fig. 2 – Subtle photoreceptors IS/OS and ELM disruptions in the eye with short duration of macular detachment.**  
IS – inner segment; OS – outer segment; ELM – external limiting membrane.

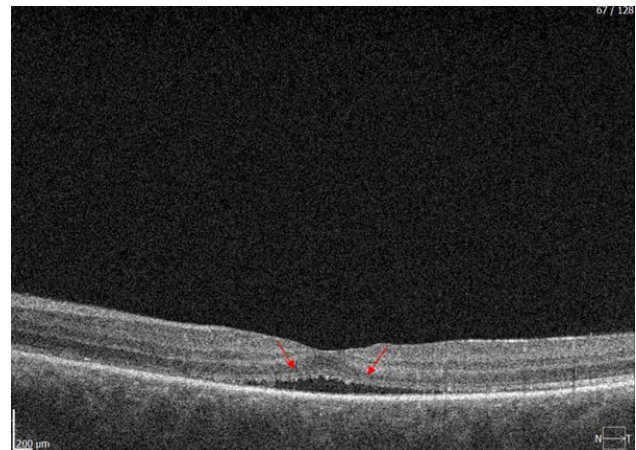
Figure 3 shows multiple zones of IS and OS photoreceptors and ELM disruptions, and RSFF in the eye with a longer duration of MD.



**Fig. 3 – Multiple areas of photoreceptors IS/OS and ELM disruptions and residual subfoveal fluid in the eye with longer duration of macular detachment.**  
IS – inner segment; OS – outer segment; ELM – external limiting membrane.

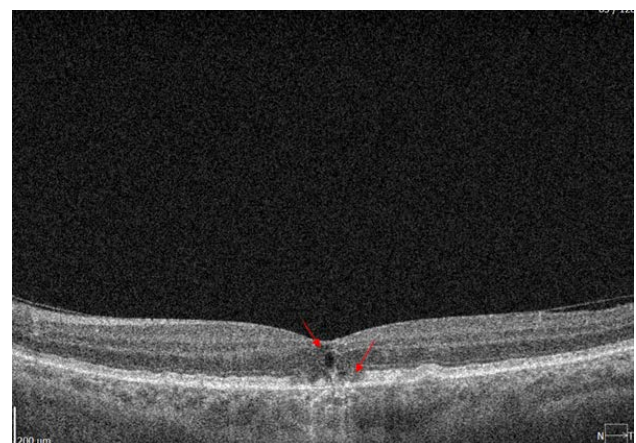
Initial postoperative SDOCT after 1 month showed persistent foveal detachment in 8 (29.6%) eyes clinically invisible on binocular funduscopic examination. On follow-up SDOCT 6 months later, SRF was noted in 3 (11.1%) eyes. At the end of the follow-up period, all eyes had complete resorption of the SRF except for a small localized subfoveal fluid (SFF) accumulation noted in 1 (3.7%) eye. In eyes with RD duration shorter than 7 days, subtle SFF was present in 2 (18.2%) eyes on initial SDOCT imaging, and all of these eyes during the follow-up period had complete SFF resolution. In eyes with RD lasting 8–14 days, RSFF was noted in 3 (37.5%) eyes and 1 (12.5%) eye on SDOCT examination

after 1 and 6 months, respectively. In the eyes with RD lasting 15–30 days, RSFF was noted in 2 (40.0%) eyes and 1 (20.0%) eye on SDOCT examination after 1 and 6 months, respectively. At the end of the follow-up period, complete resolution of SRF occurred in all of these eyes. In the eyes with RD for more than one month, RSFF was present in 1 (33.3%) eye throughout the whole follow-up period. There was a statistically significant difference in the incidence of RSFF between the eyes depending on the duration of MD ( $p = 0.041$ ). Figure 4 shows persistent RSFF in operated eyes.



**Fig. 4 – Residual persistent subfoveal fluid in operated eyes.**

Cystic changes after retinal reattachment in the retinal layers were found in 4 (14.8%) eyes, 2 (7.4%) eyes, and 1 (3.7%) eye on OCT examination after 1, 6, and 12 months, respectively. Figure 5 shows cystic spaces in the reattached retina with areas of IS and OS photoreceptors and ELM disruptions.



**Fig 5. - Cystic spaces in reattached retina with areas of photoreceptors IS/OS and ELM disruptions.**  
IS – inner segment; OS – outer segment; ELM – external limiting membrane.

ELM after retinal reattachment was detected in only 2 (7.4%) eyes on SDOCT examination after 6 and 12 months, and all of these eyes belonged to the group of eyes with a duration of RD longer than one month.

## Discussion

A variety of histopathological changes occur during RD that may contribute to low functional success rates following retinal reattachment. Experimental RD models have shown degenerations of ORL<sup>8,9,16</sup>. RD primarily affects the photoreceptor layer, and the majority of the changes occur in this retinal layer. The central part of the macula, *fovea centralis*, mostly depends on the supply of oxygen and nutrients from the choroidal circulation<sup>17</sup>. The first pathologic changes in OS of the photoreceptors during RD occur due to loss of nutrition in ORL. During RD, OS and synaptic terminals of the photoreceptors and photoreceptor bodies in the ONL degenerate<sup>18</sup>.

Even a short duration of RD can induce significant histological changes. The apoptosis of the photoreceptor can occur as early as 1–3 days after experimental RD<sup>19</sup>. In animal experiments, retinal degeneration tends to worsen as the detachment period increases<sup>20</sup>. Prolonged RD causes apoptosis and necrosis of the photoreceptors and ONL complex. These changes are irreversible<sup>21</sup>. Therefore, the patients may have limited visual function recovery despite successful RD repair and the absence of biomicroscopically visible alterations because of irreversible damage of photoreceptors via apoptosis which may have occurred before RD repair<sup>9</sup>. Loss and degeneration of photoreceptors lead to a reduction in retinal thickness. In the current study, the mean TRT of the normal eye was approximately 314  $\mu\text{m}$ , while the mean TRT of affected eyes was nearly 15  $\mu\text{m}$  less, demonstrating a mean reduction of 5%. In terms of average ORT, this thinning was also observed. In our study, the mean ORT of the normal eye was approximately 152  $\mu\text{m}$ , while the mean ORT of affected eyes was nearly 10  $\mu\text{m}$  less, demonstrating a mean reduction of 6.5%.

The overall decrease in the mean macular thickness is mainly the result of decreases in the thicknesses of ORL, including PRL, OPL, and ONL. In our study, we did not notice differences in the thicknesses of ORL among eyes with an MD duration of up to 2 weeks. However, notable additional decreases in the thickness of ORL were observed in the eyes with a longer detachment period. In our study, we revealed significant thinning of PRL and ONL in reattached retina even in the eyes with a mean duration of RD longer than 2 weeks. The thickness of outer retinal structures significantly decreased after detachment over time and correlated with the duration of RD. The significant thinning of ORL observed in the reattached region in our work agrees with the findings of previous studies<sup>22,23</sup>.

The structural integrity of the PRL and ONL are closely associated with visual function. Based on the previous studies, repair of a detached macula may be delayed up to 1 week without significant adverse effects on vision outcome<sup>24,25</sup>. Early intervention is needed because long-term structural changes may remain despite only a few days of prolonged detachment duration. Thus, the structural changes found in our study may highlight the importance of initial injury to the retinal tissue, such as apoptosis<sup>21</sup>.

Previous studies with 5- to 6-month follow-up periods after retinal reattachment showed gradual lengthening of the photoreceptor OS in animals with a detachment period of less than 7 days<sup>16,26</sup>. Following retinal reattachment, the PRL regenerates and reestablish connections with RPE. Experimental models of RD have shown that the thickness of the outer retina increases progressively and almost normalizes after approximately 150 days after reattachment<sup>18,19</sup>. In our study, in the eyes with a detachment period shorter than 2 weeks, the length of the photoreceptor OS was almost normalized at the end of the follow-up period.

With improved SDOCT technology (axial resolution of 3  $\mu\text{m}$  to 5  $\mu\text{m}$ ), IS and OS photoreceptor disruptions were reported by several groups after the successful repair of RRD<sup>27,28</sup>. On SDOCT scans, the abrupt boundary between the IS and OS junction is recognized as the back reflection. Recently, it has been suggested that the band, often attributed to the boundary between the IS and OS of the photoreceptors, may align with the ellipsoid portion of the inner segments. In this sense, the disrupted ellipsoid zone could be a marker of poor prognosis for vision recovery<sup>12,29</sup>. Furthermore, ELM disruptions on SDOCT may indicate damage to the photoreceptor's nuclear bodies with their irreversible loss<sup>4,7,11,30</sup>. In our study, in the eyes with the RD duration longer than 2 weeks before the surgery, the initial SDOCT scans acquired one month after repair of RD revealed multiple areas of IS and OS photoreceptor and ELM disruption located in the *fovea centralis*. This finding may explain the very modest vision recovery in these eyes despite successful anatomical repair.

Experimental models have shown that RD primarily affects the ORL while, at the same time, the IRL are minimally altered<sup>31</sup>. Only a few examiners have investigated the condition of the IRL after successful postoperative retinal reattachment, and they have reported mostly opposite results. In some studies, the thickness of IRL was normal, while other studies have shown a reduction in the IRT after successful surgery<sup>32–34</sup>. We evaluated NFL, GCL, and IPL thickness and found that these values were minimally lower in the eyes that underwent surgery than in the fellow eyes, and the difference was not statistically significant. It seems that neither the RD nor the surgical SB procedure significantly affects the thinning of the inner layers of the retina. In contrast, numerous studies have demonstrated a significant GCL and NFL thinning following PPV with air or silicone oil tamponade. It may be associated with additional traumatizing factors related to PPV itself, such as the surgical removal of the vitreous with loss of its protective and antioxidant function<sup>31,35</sup>.

Numerous previous studies have shown that after successful SB surgery, an RSRF in the *fovea centralis* can exist very often, probably due to poor adherence between the neurosensory retina and RPE. The persistent RSFF causes prolonged foveal detachments, and that may be responsible for the poor recovery of VA<sup>5,36</sup>. The etiology of localized foveal detachment in patients after successful RD surgery is not clear, but it is presumed to be related to a disturbance in choroidal circulation<sup>37,38</sup>. Several studies have indicated that the

incidence of persistent fluid is higher if the encircling band is used during surgery. The placement of the cerclage performs circular compression in the entire equatorial zone of 360 degrees, which can disrupt choroidal circulation to a much greater extent than when only segmental scleral explant is used. The venous congestion and reduced drainage of the choroidal circulation can be caused by the overly tight encircling band. In addition, the use of cryotherapy may be responsible for the development of choroidal inflammation, which may lead to subfoveal choroidal thickening<sup>39</sup>. In our work, delayed SRF absorption on binocular funduscopy examination was not noted. However, initial postoperative SDOCT after 1 month revealed persistent foveal detachment in 8 eyes (29.6%), which was not clinically visible on binocular funduscopy examination. In our study, a high frequency of SRF in the early postoperative period can be explained by the fact that the encircling band was used in all patients. Initially, persistent SRF was believed to lead to a permanent reduction in BCVA after RD repair. However, studies that examined eyes with SRF over a longer period of follow-up showed that in most cases after SB surgery, there was complete resorption of SRF after 6 to 24 months without significant impact on the final visual function. Foveal detachment usually improves with time, and spontaneous resolution of SRF may take up to 12 months with corresponding postoperative improvement in VA. That could be explained by remodeling and adaptation of the choroidal vascular drainage<sup>40</sup>. All our patients had complete resolution of the SRF at the end of the follow-up period except for the small localized SRF accumulation noted in 1 eye (3.7%).

CME is the formation of cystic spaces within the retinal tissue, and in longstanding cases, it may disrupt the photoreceptors and other layers of the retina and cause delayed or limited vision recovery. After SB surgery, the incidence of macular edema (whether cystoid or diffuse) has been reported by other studies with a wide range of incidence of 1.58–67%<sup>41, 42</sup>. In our study, cystic changes in the retinal layers were found only in 4 eyes after 1 month, 2 eyes after 6 months, and 1 eye at the end of the follow-up period.

We found EMM in only 2 eyes (7.4%) on SDOCT examination after 6 and 12 months, and all of these eyes belonged to the group of eyes with a duration of RD longer than 1 month. That is significantly lower than the values recorded in the eyes that underwent PPV vitrectomy. Wakabayashi et al.<sup>12</sup> observed ELM in 23% of their patients who underwent PPV<sup>43</sup>. This is likely due to differences in surgical techniques used in their studies. Apart from using cryopexy, they also used endolaser, silicone, or intravitreal gas tamponade, which could have led to an increase in the frequency of ELM in their patients.

According to available literature data, the SB procedure is a relatively safe surgical technique that does not itself change the microstructure of the macula, except for the changes in the choroidal circulation described above that may be the cause of prolonged RSFF.

Duration of RD is a well-confirmed predictive factor for vision gain after surgery<sup>44</sup>. The complete reattachment of the retina and macula does not necessarily imply a full return

of visual function. Reattachment of the retina does not in itself guarantee a complete recovery of macular function. Many patients may have limited vision recovery despite successful RD repair and biomicroscopically invisible retinal abnormalities, indicating irreversible damage of photoreceptors via apoptosis which may have occurred before RD repair.

The postsurgical VA gain is mostly influenced by the duration and extent of the MD and postoperative macular changes<sup>1, 2, 9, 18</sup>. In the eyes with detached macula preoperatively, only 37% achieve 0.4 or better VA despite an anatomic success rate of 90%<sup>3</sup>. The results of our study are in complete agreement with these findings. In our work, even in the eyes with a duration of MD of less than 7 days, VA gain was limited and at the end of the follow-up period mean BCVA in these eyes was  $0.55 \pm 0.12$ . The postoperative VA correlated negatively with the duration of MD. In our work, there was a statistically significant difference in postoperative VA between the eyes with a duration of preoperative MD shorter than 2 weeks and the eyes with a longer MD throughout the follow-up period. The time within 2 weeks appeared to be critical for macular recovery. Our work has shown that if the macula was detached for several weeks, vision recovery would generally be worse, and postsurgical VA, despite reattachment, would range only between 0.1 and 0.2. All this indicates that photoreceptor damage via apoptosis occurs early during RD and tends to worsen as the detachment period increases<sup>19, 20</sup>.

The disruption of photoreceptors IS and OS, and ELM abnormalities are strongly associated with VA in the early postoperative period and the longer follow-up. Our work confirmed that the integrity of the photoreceptor IS and OS junction is an important factor for vision restoration in the eyes after the repair of macula-involving RD.

In our study, 15 eyes with postoperative BCVA of 20/60 or more had intact IS and OS junctions on initial imaging. Moreover, our results showed a statistically significant difference in the frequency of disruption of the photoreceptor IS and OS, and ELM depending on the preoperative detachment duration ( $p = 0.007$ ). In the eyes with MD duration of up to 2 weeks, these photoreceptor changes resolved completely during the follow-up period. One year after surgery, the final SDOCT showed complete restoration of IS and OS junction that correlated with improvement in BCVA. The eyes with persistent IS and OS disruptions (mostly the eyes with a preoperative duration of RD longer than a month) had persistently poor or worsening BCVA (0.02–0.03) at 1-year follow-up. In these eyes, the postoperative SDOCT scans made one year after surgery showed permanent IS and OS junction disruptions in the subfoveal region which in some eyes were even more pronounced compared to earlier SDOCT recordings.

Our results suggest a conclusion that does not differ from the conclusions of the previously published studies. Namely, a case with a macula-off RD must receive surgical treatment within a week in order to prevent irreversible changes in macular structure and achieve the highest improvement of VA.

The limitations of our study include the relatively small number of eyes analyzed and the fact we had to rely on the anamnesis of the examined patients about the length of the preoperative macula-off period, which may not be accurate to estimate its exact duration. More comprehensive studies are needed to investigate further the effect of macula-off duration on macular morphological changes and VA.

### Conclusion

The photoreceptor degeneration via apoptosis occurs early after RD and leads to a reduction in retinal thickness. The overall decrease in the average retinal thickness after successful anatomical repair of RD is the result of the decrease in the thickness of ORL, including ONL, OPL, and PRL.

In the eyes, after the anatomically successful repair of RD, the alterations of the ELM and IS and OS of photoreceptors observed on the early SDOCT scans are mostly associat-

ed with limited vision recovery. The presence of SRF on the early postoperative SDOCT images may lead to a transient decrease in visual function, but it usually appears to resolve spontaneously with long-term follow-up. The postsurgical VA gain is mostly influenced by the duration and extent of the MD. The prolonged MD leads to damage of the neurosensory tissue of the retina and especially the photoreceptors, which may explain the limited vision recovery after successful SB surgery RD repair. In order to determine possible mechanisms for vision recovery, additional clinical and histological studies are required to evaluate the long-term macular morphologic and functional changes after successful RD and MD repair.

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### R E F E R E N C E S

- Burton TC. Recovery of visual acuity after retinal detachment involving the macula. *Trans Am Ophthalmol Soc* 1982; 80: 475–97.
- Kusaka S, Toshino A, Ohashi Y, Sakaue E. Long-term visual recovery after scleral buckling for macula-off retinal detachments. *Jpn J Ophthalmol* 1998; 42(3): 218–22.
- Pastor JC, Fernández I, Rodríguez de la Rúa E, Coco R, Sanabria-Ruiz Colmenares MR, Sánchez-Chicharro D, et al. Surgical outcomes for primary rhegmatogenous retinal detachments in phakic and pseudophakic patients: the Retina 1 Project—report 2. *Br J Ophthalmol* 2008; 92(3): 378–82.
- Cheng KC, Cheng KY, Cheng KH, Chen KJ, Chen CH, Wu WC. Using optical coherence tomography to evaluate macular changes after surgical management for rhegmatogenous retinal detachment. *Kaohsiung J Med Sci* 2016; 32(5): 248–54.
- Wolfensberger TJ, Gonvers M. Optical coherence tomography in the evaluation of incomplete visual acuity recovery after macula-off retinal detachments. *Graefes Arch Clin Exp Ophthalmol* 2002; 240(2): 85–9.
- Ricker LJ, Noordzij LJ, Goetzinne F, Cals DW, Berendschot TT, Liem AT, et al. Persistent subfoveal fluid and increased preoperative foveal thickness impair visual outcome after macula-off retinal detachment repair. *Retina* 2011; 31(8): 1505–12.
- Farooq S, Mahsood YJ, Jan S. Optical Coherence Tomography: Macular findings after successful sclera buckling in eyes with compromised visual status. *J Coll Physicians Surg Pak* 2018; 28(4): 297–300.
- Sakai T, Calderone JB, Lewis GP, Linberg KA, Fisher SK, Jacobs GH. Cone photoreceptor recovery after experimental detachment and reattachment: an immunocytochemical, morphological, and electrophysiological study. *Invest Ophthalmol Vis Sci* 2003; 44(1): 416–25.
- Lewis GP, Charteris DG, Sethi CS, Leitner WP, Linberg KA, Fisher SK. The ability of rapid retinal reattachment to stop or reverse the cellular and molecular events initiated by detachment. *Invest Ophthalmol Vis Sci* 2002; 43(7): 2412–20.
- Barr CC. The histopathology of successful retinal reattachment. *Retina* 1990; 10(3): 189–94.
- Rashid S, Pili S, Chin EK, Zawadzki RJ, Werner JS, Park SS. Five-year follow-up of macular morphologic changes after rhegmatogenous retinal detachment repair: Fourier domain OCT findings. *Retina* 2013; 33(10): 2049–58.
- Wakabayashi T, Oshima Y, Fujimoto H, Murakami Y, Sakaguchi H, Kusaka S, et al. Foveal microstructure and visual acuity after retinal detachment repair: Imaging analysis by Fourier-domain optical coherence tomography. *Ophthalmology* 2009; 116(3): 519–28.
- Kim JH, Park DY, Ha HS, Kang SW. Topographic changes of retinal layers after resolution of acute retinal detachment. *Invest Ophthalmol Vis Sci* 2012; 53(11): 7316–21.
- Arroyo JG, Yang L, Bula D, Chen DF. Photoreceptor apoptosis in human retinal detachment. *Am J Ophthalmol* 2005; 139(4): 605–10.
- Alam S, Zawadzki RJ, Choi S, Gerth C, Park SS, Morse L, et al. Clinical application of rapid serial Fourier-domain optical coherence tomography for macular imaging. *Ophthalmology* 2006; 113(8): 1425–31.
- Guerin CJ, Anderson DH, Fariss RN, Fisher SK. Retinal reattachment of the primate macula. Photoreceptor recovery after short-term detachment. *Invest Ophthalmol Vis Sci* 1989; 30(8): 1708–25.
- Faude F, Francke M, Makarov F, Schuck J, Gärtner U, Reichelt W, et al. Experimental retinal detachment causes widespread and multilayered degeneration in rabbit retina. *J Neurocytol* 2001; 30(5): 379–90.
- Lewis GP, Charteris DG, Sethi CS, Fisher SK. Animal models of retinal detachment and reattachment: identifying cellular events that may affect visual recovery. *Eye (Lond)* 2002; 16(4): 375–87.
- Guerin CJ, Lewis GP, Fisher SK, Anderson DH. Recovery of photoreceptor outer segment length and analysis of membrane assembly rates in regenerating primate photoreceptor outer segments. *Invest Ophthalmol Vis Sci* 1993; 34(1): 175–83.
- Cook B, Lewis GP, Fisher SK, Adler R. Apoptotic photoreceptor degeneration in experimental retinal detachment. *Invest Ophthalmol Vis Sci* 1995; 36(6): 990–6.
- Murakami Y, Notomi S, Hisatomi T, Nakazawa T, Ishibashi T, Miller JW, et al. Photoreceptor cell death and rescue in retinal detachment and degenerations. *Prog Retin Eye Res* 2013; 37: 114–40.
- Takkar B, Azad R, Kamble N, Azad S. Retinal nerve fiber layer changes following primary retinal detachment repair with silicone oil tamponade and subsequent oil removal. *J Ophthalmic Vis Res* 2018; 13(2): 124–9.



23. *Akkoyun I, Yılmaz G.* Optical coherence tomography: Anatomic and functional outcome after scleral buckling surgery in macula-off rhegmatogenous retinal detachment. *Klin Monbl Augenheilkd* 2013; 230(8): 814–9. (German)
24. *Diederer RM, La Heij EC, Kessels AG, Goezinne F, Liem AT, Hendrikse F.* Scleral buckling surgery after macula-off retinal detachment: worse visual outcome after more than 6 days. *Ophthalmology* 2007; 114(4): 705–9.
25. *Liu F, Meyer CH, Mennel S, Hoerle S, Kroll P.* Visual recovery after scleral buckling surgery in macula-off rhegmatogenous retinal detachment. *Ophthalmologica* 2006; 220(3): 174–80.
26. *Anderson DH, Guerin CJ, Erickson PA, Stern WH, Fisher SK.* Morphological recovery in the reattached retina. *Invest Ophthalmol Vis Sci* 1986; 27(2): 168–83.
27. *Lai WW, Leung GY, Chan CW, Yeung IY, Wong D.* Simultaneous spectral domain OCT and fundus autofluorescence imaging of the macula and microperimetric correspondence after successful repair of rhegmatogenous retinal detachment. *Br J Ophthalmol* 2010; 94(3): 311–8.
28. *Sheth S, Dahir S, Natarajan S, Mbatre A, Labauri N.* Spectral domain-optical coherence tomography study of retinas with a normal foveal contour and thickness after retinal detachment surgery. *Retina* 2010; 30(5): 724–32.
29. *Rossetti A, Doro D, Manfrè A, Midena E.* Long-term follow-up with optical coherence tomography and microperimetry in eyes with metamorphopsia after macula-off retinal detachment repair. *Eye* 2010; 24(12): 1808–13.
30. *Spaide RF, Curcio CA.* Anatomical correlates to the bands seen in the outer retina by optical coherence tomography. *Retina* 2011; 31(8): 1609–19.
31. *Gharbiya M, Albanese GM, Plateroti AM, Marcelli M, Marengo M, Lambiase A.* Macular ganglion cell layer thickness after macula-off rhegmatogenous retinal detachment repair: scleral buckling versus pars plana vitrectomy. *J Clin Med* 2020; 9(5): 1411.
32. *Lee SH, Han JW, Byeon SH, Kim SS, Koh HJ, Lee SC, et al.* Retinal layer segmentation after silicone oil or gas tamponade for macula-on retinal detachment using optical coherence tomography. *Retina* 2018; 38(2): 310–9.
33. *Lee YH, Lee JE, Shin YI, Lee KM, Jo YJ, Kim JY.* Longitudinal changes in retinal nerve fiber layer thickness after vitrectomy for rhegmatogenous retinal detachment. *Invest Ophthalmol Vis Sci* 2012; 53(9): 5471–4.
34. *Menke MN, Kowal JH, Dufour P, Wolf-Schnurrbusch UE, Ceklic L, Framme C, et al.* Retinal layer measurements after successful macula-off retinal detachment repair using optical coherence tomography. *Invest Ophthalmol Vis Sci* 2014; 55(10): 6575–9.
35. *Ozdek S, Lonneville Y, Onol M, Gurelik G, Hasanreisoglu B.* Assessment of retinal nerve fiber layer thickness with NFA-GDx following successful scleral buckling surgery. *Eur J Ophthalmol* 2003; 13(8): 697–701.
36. *Hagimura N, Iida T, Suto K, Kishi SL.* Persistent foveal retinal detachment after successful rhegmatogenous retinal detachment surgery. *Am J Ophthalmol* 2002; 133(4): 516–20.
37. *Yoshida A, Hirokawa H, Ishiko S, Ogasawara H.* Ocular circulatory changes following scleral buckling procedures. *Br J Ophthalmol* 1992; 76(9): 529–31.
38. *Takabashi K, Kishi S.* Remodeling of choroidal venous drainage after vortex vein occlusion following scleral buckling for retinal detachment. *Am J Ophthalmol* 2000; 129(2): 191–8.
39. *Lira RP, Takasaka I, Arieta CE, Nascimento MA, Caldato R, Panetta H.* Cryotherapy vs laser photocoagulation in scleral buckle surgery: A randomized clinical trial. *Arch Ophthalmol* 2010; 128(12): 1519–22.
40. *Kimura M, Nishimura A, Yokogawa H, Okuda T, Higashide T, Saito Y, et al.* Subfoveal choroidal thickness change following segmental scleral buckling for rhegmatogenous retinal detachment. *Am J Ophthalmol* 2012; 154(5): 893–900.
41. *Lee SY, Joe SG, Kim JG, Chung H, Yoon YH.* Optical coherence tomography evaluation of detached macula from rhegmatogenous retinal detachment and central serous chorioretinopathy. *Am J Ophthalmol* 2008; 145(6): 1071–6.
42. *Zhou C, Lin Q, Chen F.* Prevalence and predictors of metamorphopsia after successful rhegmatogenous retinal detachment surgery: a cross-sectional, comparative study. *Br J Ophthalmol* 2017; 101(6): 725–9.
43. *Gibran SK, Cleary PE.* Ocular coherence tomographic examination of postoperative foveal architecture after scleral buckling vs vitrectomy for macular off retinal detachment. *Eye (Lond)* 2007; 21(9): 1174–8.
44. *Williamson TH, Shunmugam M, Rodrigues I, Dogramaci M, Lee E.* Characteristics of rhegmatogenous retinal detachment and their relationship to visual outcome. *Eye (Lond)* 2013; 27: 1063–9.

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## Gender differences in suicide in Serbia within the period 2016–2020

Polne razlike osoba koje su izvršile samoubistvo u Srbiji u periodu od 2016. do 2020. godine

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### Abstract

**Background/Aim.** About one million suicide deaths occur worldwide annually, which indicates the importance of this problem. The aim of the study was to give an overview of the status of gender differences in suicides committed in Serbia from 2016 to 2020 and to highlight the most important trends over the past ten years. This investigation continues the previous one from the period 2011–2016. **Methods.** The same method was used as in previous investigations with data obtained from the Statistical Office of the Republic of Serbia, estimating gender differences for the total number of suicides, suicide rates, sociodemographic characteristics of suicidal victims, and methods of suicide. **Results.** Within the period 2016–2020, 4,752 suicides in Serbia were committed; among them, 75.1% were males (m) and 24.9% females (f) – m : f = 3 : 1. The annual suicide rate showed a constant decrease from 2016 to 2020, and in 2020, it was the lowest (13 per 100,000 inhabitants). Married men (43.4%) and widowed women (38.6%), retired, with secondary education most often committed suicide. About a quarter (24.5%) of suicide committers were older than 75, and 42.6% were older than 65 years. Hanging, strangulation, and suffocation were the most common suicide methods – 64% (m) and 54.9% (f). **Conclusion.** In the last decade, the suicide rate continued to decrease in Serbia year by year. Within the period 2016–2020, the m/f ratio of suicide rate in the age differences varied from 2.4 in the oldest group (older than 75 years) to 9.1 in the group of adolescents. The Suicide Prevention Program in Serbia should be primarily targeted at two age groups at the highest risk of committing suicide, the old adult population and male adolescents. A comprehensive state prevention program should include education for general practitioners for early detection of high-risk individuals to provide them with psychiatric care, including support for suicide survivors.

### Key words:

aged; epidemiology; gender identity; risk factors; serbia; suicide.

### Apstrakt

**Uvod/Cilj.** U svetu se izvrši oko milion samoubistava godišnje, što ukazuje na značaj tog problema. Cilj rada bio je da se prikažu polne razlike kod osoba koje su izvršile samoubistvo u Srbiji u periodu od 2016. do 2020. godine i da se ukaže na najvažnije trendove u prethodnih 10 godina. Rad je nastavak istraživanja samoubistva u Srbiji za period od 2011. do 2016. godine. **Metode.** U istraživanju je korišćena jednaka metoda kao i u prethodnim istraživanjima, sa podacima dobijenim od Zavoda za statistiku Republike Srbije, procenom polne razlike za ukupan broj i stopu samoubistava, sociodemografskih karakteristika žrtava samoubistava i metoda samoubistava. **Rezultati.** U periodu od 2016. do 2020. ukupan broj samoubistava u Srbiji iznosio je 4 752, od čega su 75,1% bili muškarci (m), a 24,9% žene (ž) – m : ž = 3 : 1. Godišnja stopa samoubistava je konstantno opadala od 2016. do 2020. godine i 2020. godine bila je najniža (13 na 100 000 stanovnika). Samoubistvo su najčešće izvršili oženjeni muškarci (43,4%) i udovice (38,6%), sa srednjim obrazovanjem, u penziji. Oko četvrtine (24,5%) samoubistava izvršile su osobe starije od 75 godina, a 42,6% osobe starije od 65 godina. Vešanje, davljenje i gušenje su bile najčešće metode samoubistva, 64% (m) i 54,9% (ž). **Zaključak.** Stopa samoubistava u Srbiji u poslednjoj deceniji je u opadanju iz godine u godinu. U periodu od 2016. do 2020. godine odnos stope samoubistva m/ž varirao je u starosnim grupama, od 2,4 u grupi starijih od 75 godina do 9,1 u grupi adolescenata. Program prevencije samoubistva u Srbiji prvenstveno treba da bude usmeren na dve starosne grupe sa najvećim rizikom od samoubistva, na grupu starih osoba i na adolescente muškog pola. Sveobuhvatni državni program prevencije trebalo bi da bude usmeren na edukaciju lekara opšte prakse za rano otkrivanje visokorizičnih pojedinaca kako bi im se pružila psihijatrijska pomoć, uključujući i podršku preživelim.

### Ključne reči:

stare osobe; epidemiologija; pol; faktori rizika; serbija; samoubistvo.

## Introduction

Around one million suicide deaths occur worldwide annually, which indicates the importance of this problem. According to the World Health Organization (WHO), in 2019, the global suicide rate was estimated at 11.6 per 100,000 inhabitants<sup>1</sup>.

There are wide geographic differences in suicide rates. Most suicides (nearly 79%) occurred in low and medium-income countries<sup>2</sup>. The core of the problem of suicide mortality has shifted from Western Europe to Eastern Europe but now seems to be shifting to Asia. According to the WHO, the biggest suicide rate in the world occurred in Eswatini (former Swaziland) (29.4 per 100,000 inhabitants) and in South Korea (28.6 per 100,000 inhabitants)<sup>1</sup>. In India, a rising trend of suicide rates has been observed over the last five decades<sup>3</sup>.

Of the total number of deaths in Europe, 1.4 % is related to suicide<sup>4</sup>.

Six of the top ten countries with the highest suicide rates internationally are from Europe<sup>4</sup>. According to the WHO, in 2019, the leading country was Lithuania, with a suicide rate of 26.1 per 100,000 inhabitants, where the highest suicide rate among males was registered (61.2 per 100,000 inhabitants). In South Korea, the top world suicide rates occur among females (22.1 per 100,000 inhabitants)<sup>1,3</sup>.

Gender differences in suicide are observed across the countries of the European Union. Therefore, death rates from suicide are four to five times greater for men (average rate 20.7 per 100,000 inhabitants) than women (average rate 4.7 per 100,000 inhabitants)<sup>5</sup>.

The male (m) – female (f) rate ratio (m/f ratio) of suicide is estimated to be the highest in the European region (m : f – 4.0 : 1), but the lowest is in the Eastern Mediterranean region<sup>2</sup>.

Among males, the highest suicide rate is observed in the following groups: Europe in the 45–59 age group, the South East Asian region in the 15–29 age group, and the Western Pacific region for ages above 60<sup>2,6</sup>.

The remarkably high suicide rate among females is observed in Southeast Asia (15–29 years) and the Western Pacific region (from age 45)<sup>2,6</sup>.

By age, although suicide attempts are more frequent among adolescents and young adults, older men and women show the highest suicide rate in almost all countries worldwide<sup>7,8</sup>.

Over the last ten years, the suicide rate in the elderly has been almost two times higher than in young people<sup>9–11</sup>. The suicide rate increased with age among persons older than 60, and the highest suicide rate is among persons aged over 70<sup>4</sup>.

According to data obtained from the Statistical Office of the Republic of Serbia - Department of Demography (SORS), the suicide rate began constantly decreasing in 2006, and this tendency continued in the following five-year period (2011–2015)<sup>12,13</sup>.

This study continues previous investigations of suicide in Serbia<sup>12,13</sup>. The aim of the study was to present gender differences in suicide mortality in Serbia during the period

between 2016 and 2020 and highlight the most important trends over the past ten years.

## Methods

The study analyzed trends of suicide in Serbia using the data from the past 5 years, from 2016 to 2020. The distribution of different important sociodemographic characteristics of suicide by gender differences, including age group, education, employment, marital status, nationality, and method of suicide, was analyzed.

As for previous investigations, data were obtained from the SORS.

This study included data on all suicides committed in Serbia (Central Serbia and Vojvodina) during the 5-year observed period (2016–2020).

Annual suicide rates per 100,000 inhabitants were calculated using the population data for the total population and for the male and female populations separately.

Classification of data related to suicide methods was defined based on the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-X Code), WHO.

## Results

It can be noted that the highest suicide rate for Serbia as a whole in the observed period was 14.3 per 100,000 inhabitants in 2017, with a decreasing tendency in the following years, and the lowest rate was 13 per 100,000 inhabitants in 2020.

The m/f ratio in the suicide rate for the total group varied from 2.88 in 2016 to 3.38 in 2020. The highest suicide rate for males in the observed period was 22.3 per 100,000 inhabitants in 2017, with a decreasing tendency in the following years, and the lowest rate was 20.3 per 100,000 inhabitants in 2020. The highest suicide rate for females in the observed period was 7.1 per 100,000 inhabitants in 2016, and the lowest was 6.0 per 100,000 inhabitants in 2020 (Table 1).

**Table 1**

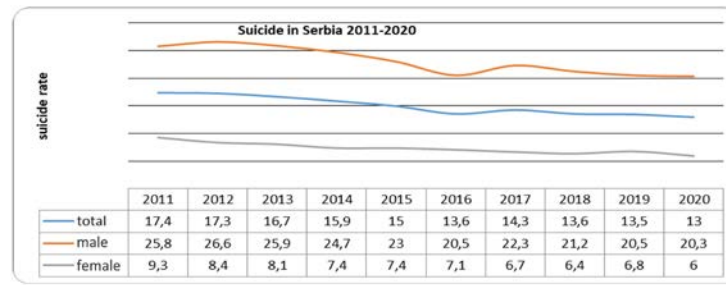
**Gender differences in annual suicide rate (SR) per 100,000 inhabitants in Serbia within the period 2016–2020**

Year of suicide	Total SR	Males SR	Females SR	m/f ratio
2016	13.6	20.5	7.1	2.88
2017	14.3	22.3	6.7	3.32
2018	13.6	21.2	6.4	3.31
2019	13.5	20.5	6.8	3.04
2020	13.0	20.3	6.0	3.38

**m/f = male/female.**

A declining trend in the suicide rate in Serbia as a whole has been noted in the last 10 years (Figure 1).

The gender differences in sociodemographic data (marital status, educational level, employment, and nationality) are shown in Table 2.



**Fig. 1 – Annual suicide rate (per 100,000 inhabitants) in males and females in Serbia within the period 2011–2020.**

**Table 2**

**Gender differences in marital status, educational level, employment, and nationality of suicide committers in Serbia within the period 2016–2020**

Socio-demographic data	2016 n	2017 n	2018 n	2019 n	2020 n	Total n (%)	m/f ratio
<b>Marital status</b>							
single							
m	180	220	180	202	185	967 (84.8)	5.59
f	47	22	37	37	30	173 (15.2)	
married							3.72
m	322	318	319	291	299	1 549 (78.8)	
f	79	100	74	83	80	416 (21.2)	
widowed							1.36
m	123	133	129	111	127	623 (57.7)	
f	105	93	85	95	79	457 (42.3)	
divorced							3.01
m	77	88	91	90	70	416 (75.1)	
f	27	27	33	27	24	138 (24.9)	
unknown	2	4	1	5	1		
<b>Educational level</b>							
no school							1.38
m	49	56	51	37	33	226 (58.1)	
f	47	31	28	30	27	163 (41.9)	
uncompleted primary school							1.97
m	64	60	57	52	55	288 (66.4)	
f	33	30	24	34	25	146 (33.6)	
primary school							2.89
m	177	192	195	181	183	928 (74.3)	
f	69	59	59	67	67	321 (25.7)	
secondary school							4.38
m	339	362	339	349	345	1734 (81.4)	
f	89	84	85	71	67	396 (18.6)	
high school							3.07
m	29	27	32	24	20	132 (75.4)	
f	9	12	6	9	7	43 (24.6)	
university							2.39
m	29	51	31	40	45	196 (70.5)	
f	7	12	22	24	17	82 (29.5)	
no data							1.85
m	17	15	15	15	1	63 (64.9)	
f	4	14	5	8	3	34 (35.1)	
<b>Employment status</b>							
employed							5.58
m	285	312	257	271	237	1362 (84.8)	
f	54	42	57	53	38	244 (15.2)	
unemployed							5.30
m	123	150	129	134	100	636 (84.1)	
f	32	17	28	24	19	120 (15.9)	
retired							2.44
m	312	351	357	312	338	1670 (71.0)	
f	154	138	125	144	122	683 (29.0)	
dependents							0.49
m	18	13	27	18	15	91 (32.9)	
f	34	44	41	30	37	186 (67.1)	
<b>Nationality</b>							
Serbs							2.91
m	594	626	602	585	577	2 984 (74.4)	
f	224	202	197	213	189	1 025 (25.6)	
Hungarians							3.68
m	33	61	55	42	52	243 (78.6)	
f	15	18	13	9	11	66 (21.4)	
Croats							2.54
m	5	10	6	5	7	33 (71.7)	
f	3	4	3	2	1	13 (28.3)	
Roma							2.86
m	7	12	7	4	10	40 (74.1)	
f	3	3	1	2	5	14 (25.9)	
Slovaks							5.75
m	13	4	5	5	6	23 (85.2)	
f	0	0	0	4	0	4 (14.8)	
Rusyns							6.67
m	7	5	4	2	2	20 (86.9)	
f	1	1	0	0	1	3 (13.1)	
Rest							3.16
m	21	25	16	26	10	98 (75.9)	
f	3	10	9	2	7	31 (24.1)	
Unknown							3.70
m	24	20	25	29	13	111 (78.7)	
f	9	4	6	11	0	30 (21.3)	

**n (%) – number (percentage) of suicide committers; m – male; f – female; m/f = male/female.**

Within the period from 2016 to 2020, less than half of the male suicide committers were married (43.4%) with secondary education (48.6%) and retired (46.8%). More than one-third of female suicide committers were widowed (38.6%) or married (35.1%) with secondary education (33.4%), and more than half of them (57.6%) were retired.

According to nationality, as expected, male and female Serbs, as the majority nationality in Serbia, committed

suicide most frequently within the observed period, followed by the Hungarians and Roma ethnic minority. Within the period 2016–2020, the number of suicides in Serbia increased with the age of the suicide committers. The number of suicides reached its highest number in subjects of both genders aged over 75 years; the m/f ratio in age differences varied from 2.4 in the oldest group (older than 75 years) to 9.1 in the group of adolescents (age 15 to 24) (Table 3).

**Table 3**  
**Gender differences in age of suicide committers in Serbia within the period 2016–2020**

Age range (years)	2016	2017	2018	2019	2020	Total	m/f ratio
	n	n	n	n	n	n (%)	
<15							3.5
m	4	/	1	2	/	7 (77.8)	
f	1	/	/	1	/	2 (22.2)	
15–24							9.1
m	19	32	24	28	15	118 (90.1)	
f	3	3	4	1	2	13 (9.9)	
25–34							4.9
m	51	85	43	54	44	277 (83.2)	
f	13	17	11	11	4	56 (16.8)	
35–44							3.5
m	104	100	82	77	91	454 (78.0)	
f	22	29	24	28	25	128 (22.0)	
45–54							3.1
m	113	107	116	110	99	545 (75.4)	
f	42	33	31	36	36	178 (24.6)	
55–64							3.1
m	139	161	81	168	134	683 (75.8)	
f	52	53	32	37	44	218 (24.2)	
65–74							2.6
m	103	131	140	104	141	619 (72.5)	
f	52	53	39	51	40	235 (27.5)	
> 75							2.4
m	170	171	164	155	158	818 (70.3)	
f	73	64	69	78	62	346 (29.7)	

n (%) – number (percentage) of suicide committers; m – male; f – female; m/f = male/female.

**Table 4**  
**Gender differences in methods of suicide committers in Serbia within the period 2016–2020**

Method of suicide	2016	2017	2018	2019	2020	Total	m/f ratio
	n	n	n	n	n	n (%)	
Self-poisoning by drugs and by exposure to liquid substances (X60-65, X68-69)							0.75
m	26	39	46	48	39	198 (42.9)	
f	53	58	52	52	49	264 (57.1)	
Hanging, strangulation, and suffocation (X70)							3.51
m	451	481	466	453	433	2284 (77.8)	
f	152	133	120	133	113	651 (22.2)	
Drowning and submersion (X71)							1.38
m	16	18	11	12	20	77 (57.9)	
f	10	13	13	9	11	56 (42.1)	
Firearm and explosive material (X72-X75)							21.0
m	150	141	125	119	53	588 (95.4)	
f	8	5	9	5	1	28 (4.5)	
Sharp or blunt object (X78, X79)							2.50
m	15	20	11	18	21	85 (71.4)	
f	9	11	2	8	4	34 (28.6)	
Jumping from a high place (X80)							1.42
m	28	37	35	30	30	160 (58.8)	
f	19	14	27	29	23	112 (41.2)	

n (%) – number (percentage) of suicide committers; m – male; f – female; m/f = male/female.

The most common method of suicide both in males (64%) and females (54.9%) was hanging, strangling, and suffocation (X70), thereby 3.5 times more often in males than in females. The second most common method of suicide in males was by firearm and explosive material (X72-X75) (16.48%).

The second most common method of suicide in females was self-poisoning by drugs and by exposure to liquid substances (X60-65, X 68-69) (22.28%) (Table 4).

## Discussion

According to the data obtained from the Statistical Office of the Republic of Serbia (Department of Demography) in the observed five-year period (2016–2020), the annual suicide rate decreased constantly from 13.6 in 2016 to 13.0 in 2020.

Comparing the suicide rate in Serbia with the suicide rate in neighboring countries of former Yugoslavia (<https://www.macrotrends.net>) for the period 2016 to 2019, it can be noticed that the suicide rate in Serbia during the observed period was lower than in Montenegro (over 21 per 100,000), Slovenia (over 19 per 100,000), and Croatia (over 16.4 per 100,000), but higher than in North Macedonia (9.4 per 100,000) and Bosnia (over 10 per 100,000). Unfortunately, no data has been recorded yet in the literature for suicide rates during the pandemic period for 2020 for countries of former Yugoslavia.

Observing the last decade year by year, it is evident that the suicide rate in Serbia continued the tendency to decrease permanently from 17.4 in 2011 to the mentioned 13.0 in 2020.

For gender differences, the trend of an increasing suicide rate among the male population compared to the female one, which began in Serbia in 2006 ( $m : f = 2.56$ ), continued in the next five-year period (2011–2015) ( $m : f = 2.77-3.11$ ) and the last five-year period (2016–2020) as well ( $m : f = 2.88-3.38$ )<sup>12, 13</sup>. In support of the previously mentioned, as documented in the literature, the number of male suicide committers has been increasing globally in the past decade, while among females, there was a fall in the number of suicide cases<sup>5</sup>.

What are the reasons that caused the decrease in the suicide rate in the Serbian population?

Behind the decrease in the suicide rate within the period 2016–2020, some factors referred to the decrease in suicide numbers per year.

According to the data obtained from the SORS, 1,145 fewer people committed suicide (20%) in the observed five-year period (2016–2020) than in the previous five-year period (2011–2015).

The transformation of demographic factors and the socioeconomic structure of the Serbian population could be one of the factors that could explain the change in the suicide rate. The total number of inhabitants in Serbia decreased by 4.96% in the observed 10-year period from 7,258,753 in 2011 to 6,899,126 in 2020<sup>13</sup>. On the other hand, the age distribution of the total population changed as well. The

number of elderly persons increased for both genders. Third, but equally important, specific aspects of Serbian society with an increase of people who migrated to other countries, especially young people, for employment and education abroad, changed the demographic structure in Serbia within the last five years.

In the year 2020, the whole world began suffering from the coronavirus disease (COVID-19) pandemic. From the beginning of the pandemic, especially during the lockdown, many people lost their jobs. Some habits were changed because some of them were forced to leave their place of work to work from home. Some of them lost their close relatives and got depressed, but some committed suicide.

It is believed that disasters, in general, have an adverse effect on people and their mental health. Although previous investigations have shown that deaths by suicide increased during the 1918–1920 influenza pandemic in the USA<sup>14</sup>, it was expected that the same would happen in the COVID-19 pandemic as well<sup>15, 16</sup>.

Observing the pandemic's impact on suicide in many countries, it can be noted that data from the literature indicate that during the COVID-19 pandemic, the suicide rate decreased in many countries, including Serbia<sup>17, 18</sup>. A study comparing data on committed suicides during the COVID-19 pandemic from 21 countries (16 high-income and five upper-middle-income countries) with the data from the pre-pandemic period shows that the number of suicides has remained largely unchanged or declined at the beginning of the pandemic compared with the number of suicides in the previous years<sup>19, 20</sup>.

Our findings of the decrease in suicide rate in 2020, during the COVID-19 pandemic, require an explanation.

One of the explanations is that families were all united during the lockdown, which caused family members to strengthen their bonds. That was explained in Durkheim's studies on social integration, explaining the apparent reduction in suicides during the war. During the lockdown, which can be observed as a traumatic event for many, people explored a widespread sense of solidarity. Applying Durkheim's social integration explanation during the crisis, we might expect a decrease in suicide numbers if the coronavirus pandemic persists in the following years<sup>21</sup>. Although the current situation with an increasing number of infected people with coronavirus led people to think pessimistically with negative predictions about the future, predicting the worst-case scenario, some people successfully adapted to the new situations, trying to rediscover themselves, their needs, their hobbies, escaping from busy daily routines, and sharing more time with their families.

People realized that if they were in a crisis, they would probably get support from their family and friends. A general feeling of solidarity in society buffered the aspect of 'I am alone' that exists in suicidal thoughts and precedes suicide act<sup>7, 22</sup>. Another equally very important explanation is that during the first and second wave of the COVID-19 infection, people in crisis could get psychological help from the SOS telephone emergency service, organized by institutions like

psychiatric hospitals and Serbian psychotherapeutic associations.

As for sociodemographic data, the trend which began in 2011 continued in the following five-year period. Differences were not observed in the sociodemographic characteristics of suicide committers according to the previous five-year period (2011–2015) because married men and widowed women with secondary school education, who were retired, most often committed suicide within the mentioned period<sup>12, 23</sup>.

After reviewing the distribution of suicide victims by their education for the last decade, it was observed that suicide has more often taken place among suicide committers in secondary school than with a lower educated group and less with high education. Compared with the previous five-year period, it seems the tendency to commit suicide is increasing among higher-educated people while decreasing among lesser-educated<sup>12, 23</sup>.

It is interesting to note that the state of being married is not a deterrent for male suicidal behavior. The state of being married does not appear to be a protective factor, but its impact on suicide differs by gender. Men are at a higher risk of suicide while they are still married. Among females, the main risk factor for suicidal death is becoming a widow, and suicidal behavior is less often triggered when they are married<sup>14, 23</sup>. In explanation, it can be noticed that suicide as a multifactorial phenomenon contains various variables which affect one individual wishing to die. Therefore, suicidal behavior is the product of the interaction of many factors, biological and psychological, not only sociodemographic, like marriage. That means that other factors, like the presence of chronic and debilitating diseases, often accompanied by profound psychological suffering, are a powerful motif for male suicide victims to commit suicide<sup>24, 25</sup>.

On the other hand, mental state is a significant risk factor for female suicide committers, mostly suffering from depression<sup>10</sup>. For women, widowhood initially causes family and friendly network support. With time passing by, this support network weakens, and the widow becomes decidedly lonely. Feeling isolated, with a sense of deep-seated sadness, women become vulnerable and emotionally emptied. Turning in on themselves, the only solution they find is suicide<sup>26</sup>.

By age, in the observed five-year period, 42.5% of suicides were committed by persons over 65, i.e., about a quarter of total suicide committers (24.5%) are persons over 75.

Suicide may occur at different stages within the life span of a person, but the elderly remains a category of the world's population at major risk of suicide.

Risk factors for elderly suicide are specific sociodemographic risk factors such as older age, marital status, and male gender. Psychological factors are also important. The most important factors are the sense of worthlessness, the feeling of social disconnectedness, and the lack of social support. Some of them, such as the loss of an emotional partner, loneliness, and bereavement (especially in

men), as well as conflicts in the family, cause great problems preceding suicide. Clinical factors like psychiatric illnesses (depression, alcohol misuse, previous suicide attempt, vulnerable personality traits, dementia, etc.) are important. Some chronic physical illnesses (pain, disability) are also connected<sup>23, 24</sup>. Sinyor et al.<sup>25</sup> identified three groups of suicide risk factors among elderly suicide committers over 80 years. The first group included marital status (married or widowed patients). The second group is connected with social connections, stressing the risk factors in individuals living alone or being socially isolated. The third group refers to health problems, including persons suffering from depression and dementia.

Suicide in the elderly population draws less attention than suicide in the younger population. It is a phenomenon that is very often ignored or neglected because elderly suicide victims are not employed and have a lower economic impact on society. Although the elderly get better health care and achieve a better quality of life and their lives are prolonged, most are a burden for their family<sup>27, 28</sup>.

In the last several decades, the 'traditional' family and the role of the elderly in the family and society have also changed. Young family members are not always ready to take care of their elderly parents, explaining they are busy and have less time to dedicate to them. All these factors make the elderly feel like a burden to their family and society.

One explanation for the higher suicide among men than women, especially among the elderly, could be that women have a greater ability to adapt to new life events. In old age, emotions tend to change in quantity and quality. Retirement, as the onset of life's third age, for some people, especially men, provokes feelings of helplessness and hopelessness. They might be anxious, worried about the future, or depressed. Women are at an advantage because they are often emotionally protected against feelings of worthlessness and self-esteem since they are occupied with children, grandchildren, and the household. In women, those small everyday matters may serve as emotional protection against feelings of worthlessness and for self-esteem<sup>29-31</sup>.

### **Suicide prevention program**

A comprehensive suicide prevention program (SPP) is generally aimed at the early detection of high-risk individuals, targeting medical service delivery to such individuals and supporting family members of suicide committers (the so-called survivors).

Medical service delivery is aimed at improving the early detection and treatment of mental illness, especially early detection of depression<sup>22, 23, 26</sup>.

The SPP in Serbia should primarily be targeted at two age groups with the highest risk of committing suicide – the old adult population (over 65 years) group and male adolescents.

With the world population constantly increasing and life expectancy becoming longer, the elderly population is becoming more prone to committing suicide. In the SPP

targeted at adults over the age of 65, as the most vulnerable population with the highest number of suicides in the last five-year period, the need to integrate specific suicide risk factors, such as psychological, feelings of social isolation, bereavement, neurocognitive impairment, as well as health problems like chronic physical illnesses and disability, should be emphasized.

Male adolescents fall in the second target group in the SPP. Like in many other countries, the suicide rate for this group is on the rise.

In SPP, the accent is on general medical professionals (GPs) because they are the first to have contact with depressed and suicidal people.

SPP include the education of GPs in improving their knowledge of depression and training in suicide crisis intervention, as well <sup>19</sup>. Therefore, healthcare professionals need to be trained to early detect depression and suicide risks. There are several reasons for that. First, older people have a higher prevalence of physical immobility, which is a reason for getting medical help but the pain is very often connected with suicidal behavior in older adults. Second, older adults are much more ready to discuss their problems with their GP but less ready to discuss their worries with their psychiatrists before suicide. In addition, it is well known that two-thirds of the elderly who committed suicide had consulted their GP in the month before suicide, half in the last 10 days. Third, some life events, such as the loss of a spouse, are considered in the elderly. Special training in crisis intervention against the development of complicated grief is recommended for the bereaved and widowed living socially isolated, followed by suicidal thoughts. In this project, social protection for the elderly must be established, followed by the government's financial support.

Further monitoring of suicide is necessary to determine the short-term and long-term consequences of the COVID-19

pandemic. At first, it includes a high-quality mental health assessment of the general population and, where appropriate, specific treatment for persons with mental illness. Second, the continued offering of medical services by SOS telephone emergency service, with an emphasis on psychiatric first aid available for people in suicidal crisis, represented a relevant mechanism for reducing the suicide rate during the COVID-19 pandemic <sup>28</sup>.

### Conclusion

In the last decade, the suicide rate in Serbia continued to decrease yearly, especially from 2016 to 2020, with the lowest being in 2020, during the COVID-19 pandemic.

Within the period from 2016 to 2020, the m/f ratio in suicide rate age differences varied from 2.4 in the oldest group (older than 75 years) to 9.1 in the group of adolescents.

SPPs in Serbia should be oriented toward two groups at the highest risk of committing suicide – primarily the elderly population and secondary male adolescents.

The suicide rate could be reduced by the government's comprehensive prevention program aimed at educating medical professionals by introducing a system for the early detection of high-risk individuals and targeting medical service delivery to such individuals, including providing support for the family members of suicide committers (the so-called survivors). The role of SOS telephone emergency service, as psychiatric first aid available for people in suicidal crisis, is also important.

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## R E F E R E N C E S

- World Health Organization. (2019). Suicide: Key Facts. [published on 2019September 2]. [accessed on 2021. January 22]. Available from: <https://www.who.int/news-room/fact-sheets/detail/suicide>
- Värnik P. Suicide in the World. *Int J Environ Res Public Health* 2012; 9(3): 760–71.
- Svain PK, Tripathy MR, Priyadarshini S, Acharya SK. Forecasting suicide rates in India: An empirical exposition. *PLoS One* 2021; 16(7): e0255342.
- Pompili M, O'Connor RC, van Heeringen K. Suicide Prevention in the European Region. *Crisis* 2020; 41(Suppl 1): S8–S20.
- Bachman S. Epidemiology of Suicide and the Psychiatric Perspective. *Int J Environ Res Public Health* 2018; 15(7): 1425.
- Conejero I, Lopez-Castroman J, Giner L, Baca-Garcia E. Sociodemographic antecedent validators of suicidal behavior: a review of recent literature. *Curr Psychiatry Rep* 2016; 18(10): 94
- Baldessarini RJ. Epidemiology of suicide: recent developments. *Epidemiol Psychiatr Sci* 2019; 29: e71.
- World Health Organization. Suicide in the world: Global Health Estimates. 2017. Available from: [http://www.who.int/mental\\_health/prevention/suicide/suicideprevent/en/](http://www.who.int/mental_health/prevention/suicide/suicideprevent/en/). [accessed 2017 September 15].
- Conwell Y, Thompson C. Suicidal behavior in elders. *Psychiatr Clin North Am* 2008; 31(2): 333–56.
- Shah AK, Bhat R, MacKenzie S, Koen C. Elderly suicide rates: cross-national comparisons of trends over a 10-year period. *Int Psychogeriatr* 2008; 20(4): 673–86.
- Crestani C, Masotti V, Corradi N, Schirripa M, Cecchi R. Suicide in the elderly: a 37-years retrospective study. *Acta Biomed* 2019; 90(1): 68–76.
- Dedić G. Gender differences in suicide in Serbia within the period 2006–2010. *Vojnosanit Pregl* 2014; 71(3): 265–70.
- Dedić G, Dedić S. Gender differences in suicide in Serbia within the period 2011–2015. *Vojnosanit Pregl* 2018; 75(12): 1165–71.
- Bonnenyn A, Shah A, Demyttenaere K. Suicidality and suicide in older people. *Rev Clin Gerontol* 2009; 19(4): 271–94.
- Miron O, Kun-Hsing Yu, Wilf-Miron R, Kohane I. Suicide Rates Among Adolescents and Young Adults in the United States, 2000–2017. *JAMA* 2019; 321(23): 2362–4.
- Abmad FB, Anderson RN. The leading causes of death in the US for 2020. *JAMA* 2021; 325(18): 1829–30.
- Rogers JP, Chesney E, Oliver D, Pollak TA, McGuire P, Fusar-Poli P, et al. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review



- and meta-analysis with comparison to the COVID-19 pandemic. *Lancet Psychiatry* 2020; 7(7): 611–27.
18. *Niederkrötenbacher T, Gunnell D, Arensman E, Pirkis J, Appleby L, Hawton K, et al.* Suicide research, prevention, and COVID-19. *Crisis* 2020; 41(5): 321–30.
  19. *Pirkis J, John A, Shin S, Del Pozo-Banos M, Arya V, Anahisa-Aguilar P, et al.* Suicide trends in the early months of the COVID-19 pandemic: an interrupted time-series analysis of preliminary data from 21 countries. *Lancet Psychiatry* 2021; 8(7): 579–88.
  20. *McIntyre R, Yena Lee Y.* Projected increases in suicide in Canada as a consequence of COVID-19. *Psychiatry Res* 2020; 290: 113104.
  21. *Devitt P.* Can we expect an increased suicide rate due to Covid-19? *Ir J Psychol Med* 2020; 37(4): 264–8.
  22. *Leske S, Kölves K, Crompton D, Arensman E, de Leo D.* Real-time suicide mortality data from police reports in Queensland, Australia, during the COVID-19 pandemic: an interrupted time-series analysis. *Lancet Psychiatry* 2021; 8(1): 58–63.
  23. *Kopp-Bigault CM.* Prevention of suicide of the elderly in France. To a multimodal strategy against depression and isolation: CQFDi. *Encephale* 2019; 45(Suppl 1): S35–S37.
  24. *Kato R, Okada M.* Can Financial Support Reduce Suicide Mortality Rates? *Int J Environ Res Public Health* 2019; 16(23): 4797.
  25. *Sinyor M, Tan LP, Schaffer A, Gallagher D, Shulman K.* Suicide in the oldest old: an observational study and cluster analysis. *Int J Geriatr Psychiatry* 2016; 31(1): 33–40.
  26. *Richard-Devantoy S, Jollant F.* Suicide in the elderly: age-related specificities? *Sante Ment Que* 2012; 37(2): 151–73. (French)
  27. *Conejero I, Olié E, Courtet P, Calati R.* Suicide in older adults: current perspectives. *Clinical Interventions in Aging* Dovepress open access to scientific and medical research. *Clin Interv Aging* 2018; 13: 691–9.
  28. *Dombrowski AY, Szanto K, Reynolds CF 3rd.* Epidemiology and risk factors for suicide in the elderly: 10-year update. *Aging Health* 2005; 1(1): 135–45.
  29. *Shah A, Bhat R, Zarate-Escudero S, DeLeo D, Erlangsen A.* Suicide rates in five-year age-bands after the age of 60 years: the international landscape. *Aging Ment Health* 2016; 20(2): 131–8.
  30. *Wasserman IM.* The impact of epidemic, war, prohibition and media on suicide: United States, 1910–1920. *Suicide Life Threat Behav* 1992; 22(2): 240–54.
  31. *Pompili M.* Can we expect a rise in suicide rates after the Covid-19 pandemic outbreak? *Eur Neuropsychopharmacol* 2021; 52: 1–2.

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## Prevalence and factors associated with depressive symptoms among medical students in their first and final year of study

Rasprostranjenost i faktori povezani sa simptomima depresije kod studenata prve i završne godine studija medicine

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### Abstract

**Background/Aim.** The mental health of medical students worsens during their studies, and research shows that medical students are at high risk of depression. The aim of this study was to evaluate the prevalence of depressive symptoms and to examine the association between various risk factors and depressive symptoms in a sample of first- and sixth-year medical students at the University of Novi Sad. **Methods.** A cross-sectional study was conducted at the Faculty of Medicine of the University of Novi Sad. The sample consisted of 308 students divided into two groups – 213 students in the first year and 95 students in the final (sixth) year of medical studies. The Patient Health Questionnaire-9 (PHQ-9) was used to measure depressive symptoms. Within this instrument, item 9 was used to identify suicidal ideation. Self-esteem was evaluated with the Rosenberg Self-Esteem Scale (RSES). Additional questions were focused on self-assessed material status, lifestyle factors, and mental healthcare-seeking. Univariate and multivariate logistic regression analyses were applied. **Results.** The prevalence of depressive episodes among medical students was 16.6%.

Almost 9% had thoughts of committing suicide. First-year students with low self-esteem were almost seven times more likely to suffer from moderate to severe depressive symptoms than those with high self-esteem. Students who had used sleeping pills or sedatives without a prescription were nearly five times more likely to have a PHQ-9 score  $\geq 10$  than those who had not [odds ratio (OR) = 4.97, 95% confidence interval (CI): 1.83–13.52]. Sixth-year students with poor or average self-assessed social relationships and low self-esteem had a stronger association with a PHQ-9 score  $\geq 10$ . **Conclusion.** There is a high prevalence of depressive episodes among first and sixth-year medical students, and it is associated with low self-esteem in both groups, use of sleeping or sedative pills without a prescription among first-year students, and poor social health in sixth-year students. We recommend routine screening for depression in medical students and establishing prevention and intervention programs.

**Key words:** depression; mental health; patient health questionnaire; signs and symptoms; students, medical; suicidal ideation.

### Apstrakt

**Uvod/Cilj.** Istraživanja pokazuju da se mentalno zdravlje studenata medicine pogoršava tokom studija i da studenti medicine imaju povećan rizik od pojave depresije. Cilj istraživanja bio je da se utvrdi rasprostranjenost simptoma depresije, kao i da se ispita povezanost simptoma depresije i različitih faktora rizika među studentima prve i šeste godine studija medicine na Univerzitetu u Novom Sadu. **Metode.** Istraživanje je sprovedeno kao studija preseka među studentima Medicinskog fakulteta Univerziteta u Novom Sadu. Uzorak je činilo 308 studenata podeljenih na dve

grupe – 213 studenata prve i 95 studenata završne (šeste) godine studiranja. Prisustvo depresivnih simptoma je ispitivano primenom Upitnika o zdravstvenom stanju bolesnika – 9 (*Patient Health Questionnaire-9*, PHQ-9), a pitanje 9 je upotrebljeno za procenu prisustva ideja o samoubistvu. Samopuzdanje je procenjavano na osnovu Rozenbergovove skale (*Rosenberg Self-Esteem Scale*, RSES). Upitnik je obuhvatio pitanja o materijalnom statusu, stilu života i korišćenju zdravstvene zaštite iz oblasti mentalnog zdravlja. Primenjena je univarijantna i multivarijantna logistička regresija. **Rezultati.** Rasprostranjenost depresivne epizode među studentima medicine bila je 16,6%. Suicidalne

ideje imalo je skoro 9% studenata. Studenti prve godine sa niskim nivoom samopouzdanja imali su skoro sedam puta veću šansu da imaju umerene do teške simptome depresije od onih sa visokim nivoom samopouzdanja. Oni koji su koristili lekove za smirenje ili lekove za spavanje koji im nisu prepisani od strane lekara imali su skoro pet puta veće šanse za PHQ-9 skor  $\geq 10$  [odds ratio (OR) = 4,97, 95% confidence interval (CI): 1,83–13,52]. Loši ili prosečni društveni odnosi i nizak nivo samopouzdanja prediktori su PHQ-9 skora  $\geq 10$  kod studenata šeste godine. **Zaključak.** Rezultati pokazuju visoku rasprostranjenost depresivne epizode kod studenata prve i šeste godine medicine, koja je povezana sa niskim

samopouzdanjem u obe grupe studenata, upotrebom lekova za spavanje ili smirenje koji nisu prepisani od strane lekara među studentima prve godine i nezadovoljavajućom socijalnom komunikacijom kod studenata šeste godine. U cilju prevencije depresije kod studenata medicine potrebno je u svakodnevnu praksu uvesti programe za otkrivanje depresije i uspostaviti preventivne i interventne programe.

**Ključne reči:** depresija; mentalno zdravlje; upitnik o zdravstvenom stanju bolesnika; znaci i simptomi; studenti medicine; samoubistvo, ideje.

## Introduction

Adolescence is often considered the healthiest time of life. Yet, there are many challenges during this life period, such as mental health problems that typically begin in late childhood and adolescence<sup>1,2</sup>.

The transition to university corresponds with this high-risk period for maladaptive coping, the onset of psychopathology, and academic failure<sup>3</sup>. Previous studies reported that at the start of medical school, the proportion of medical students suffering from psychological distress is similar to that of the general population<sup>4,5</sup>. Still, many studies suggest that the mental health of students worsens during medical training, which affects their quality of life<sup>6-9</sup> due to an unknown environment, demanding medical curriculum, excessive workload, examination pressures, difficulties with studying and time management, fear of failure, sleep deprivation, difficult patients, poor learning environments, financial concerns, information overload, and career planning<sup>10,11</sup>.

Therefore, medical education is viewed as stressful, as it is characterized by many psychological, social, and cultural changes in the life of students<sup>12</sup>.

Undesirable coping mechanisms such as substance abuse, alcohol consumption, smoking, and harm to self and others may be observed in mentally and emotionally distressed medical students<sup>13</sup>.

Medical students are suffering from various mental health problems. These include psychological stress, anxiety, depression, sleep pattern disorders, burnout, eating disorders, and potentially hazardous alcohol use<sup>14</sup>. Depression is documented as one of the risk factors most likely to lead to suicidal thinking<sup>15,16</sup>.

Rotenstein and colleagues<sup>17</sup> reported that the prevalence of current depression or depressive symptoms in medical students was 27.2%, and the prevalence of suicidal ideation was 11.1%. Results showed that the prevalence did not significantly differ between studies of either preclinical or clinical students. On the other hand, Dyrbye et al.<sup>18</sup> suggest that the prevalence of depression varies depending on the age of medical students and the stage of medical training.

The survey conducted in Serbia among medical students revealed a high rate of moderate to severe depression (22.1%), but without information concerning different levels

of education<sup>19</sup>. Another study in Serbia obtained results of medical students' testing that lasted for ten years, and it showed that the greatest portion of the examined sample (77.24%) had no depressive symptoms<sup>20</sup>.

Royal College of Psychiatrists concluded that demographic and social changes greatly influence students' mental health; therefore, it is hard to generalize earlier epidemiological studies to the present population of students<sup>21</sup>.

To get novel information, the objectives of this study were to examine and compare, among a sample of first- and sixth-year medical students at the University of Novi Sad, Serbia, the prevalence of depressive symptoms and suicidal ideation and examine the association between various risk factors and depressive symptoms.

## Methods

The research represents a cross-sectional study conducted at the Faculty of Medicine of the University of Novi Sad, Serbia, in 2019. The Ethics Committee of the Institute of the Public Health of Vojvodina, Novi Sad, approved the study (No.01-340/2). All of the respondents agreed to participate in the study. Students completed the questionnaire via anonymous online distribution with electronic consent. The sample consisted of two groups – 213 students in the first year of medical studies (response rate 96.4%) and 95 in the final (sixth) year of medical studies (response rate 47.1%).

The survey questionnaire consisted of the following parts: questions on demographics (gender, age, and year of study), socioeconomic conditions (self-assessed material status), the Patient Health Questionnaire-9 (PHQ-9)<sup>22</sup>, self-assessed social health, the Rosenberg Self-Esteem Scale (RSES)<sup>23</sup>, lifestyle factors (smoking, alcohol use, marijuana use, ecstasy use, sedative or sleeping pills use without a prescription), and mental healthcare-seeking (visit to psychiatrist/psychologist in the last 12 months).

Symptoms of depression in the previous two weeks were evaluated using the PHQ-9, which consists of nine items. Each item has a four-point severity scale ranging from "0 = not at all" to "3 = nearly every day". A total score can range from 0 to 27. A score from 0 to 4 represents no significant depressive symptoms; a score from 5 to 9 represents mild depressive symptoms; a score from 10 to 27 represents a depressive episode: moderate (10–14), moderately severe

(15–19), and severe (20–27)<sup>22</sup>. A cut-off score of 10 or above can be used regardless of age<sup>24</sup>. The PHQ-9 has been found to have good psychometric properties amongst university students because of its validity, reliability, brevity, and ease of administration<sup>25,26</sup>. Within this instrument, item 9 is sometimes referred to as the PHQ-9 suicide question because it specifically evaluates the frequency of passive thoughts of death or self-harm within the last two weeks. Participants were asked about suicidal thoughts and behaviors (“thoughts that you would be better off dead, or thoughts of hurting yourself in some way”). The presence of suicidal ideation in the past two weeks was confirmed if the respondent answered at least ‘on several days’. It is used as a single scale to assess the prevalence of suicidal ideation in research<sup>27</sup>, and the response to this item identifies outpatients at increased risk of suicide attempt or death<sup>28</sup>.

This study evaluated self-esteem with the RSES, a self-rating scale consisting of 10 items, five positively worded and five negatively worded items, using a 4-point Likert scale to rate, with options ranging from “1 = strongly disagree” to “4 = strongly agree”. Negative statements, which measure a person’s negative feelings about themselves, were reverse-scored. The total score ranged from 10 points to 40 points. Higher scores indicated higher self-esteem<sup>23</sup>, and a score lower than 30 shows low self-esteem<sup>29</sup>. This scale has good internal consistency, and it has been proven helpful in studying self-esteem among students<sup>30</sup>.

Participants were asked to evaluate alcohol use and frequency of binge drinking (defined as having six or more drinks on one occasion)<sup>31</sup>. A “smoker” was considered someone currently using cigarettes (daily, a few days a week, or less). Ever tobacco smokers were respondents who smoked cigarettes during their lifetime but not during the survey time.

### *Statistical analysis*

The categorical variables (gender, socioeconomic condition, lifestyle factors, mental health care seeking, depressive symptoms, and self-esteem) were presented with numbers and percentages. The continuous variable (age) was presented as means and standard deviation (SD). Kolmogorov–Smirnov test was conducted to indicate whether the data followed a normal distribution, and differences in the investigated variables were assessed using the Chi-squared test. To determine the possible predictive variables for medical students’ depressive symptoms, univariate and multivariate logistic regression models were implemented. PHQ-9 score as a dependent variable was transformed into a dichotomous variable. Only variables found to be statistically significantly associated with a PHQ-9 score  $\geq 10$  in univariate analysis were included in multivariate models. The following variables were tested in the multivariate models: self-assessed material status, self-assessed social health, self-esteem scale, and sleeping pills/sedative use without a prescription for first-year students and self-assessed social health, self-esteem scale, and sleeping pills/sedative use without a prescription for sixth-year students. We calculated the associa-

tion through odds ratio (OR) with 95% confidence intervals (95% CI). The probability,  $p < 0.05$ , was taken as the minimum level of significance. All the statistical analyses were performed with the SPSS 21.0 statistical package.

### **Results**

The study included 308 students from the Faculty of Medicine University of Novi Sad, Serbia. The average age of participants was 20.95 years. In both observed groups, females were more prevalent. There were more sixth-year than first-year students with a low level of self-esteem. Compared with the first-year students, sixth-year students of medicine reported more frequently that they are current smokers, used cannabis at least once in their lifetime, and used tranquilizers or sedatives without a doctor’s prescription. The results indicated a significant difference in alcohol use between first and sixth-year students. There were significantly fewer sixth-year students who had never consumed alcohol (6.3% vs. 17.4%) and had never been excessively drunk (36.8% vs. 53.5%) compared to first-year students. One in ten students visited a psychiatrist or psychologist in the previous 12 months. The characteristics of the sample are summarized in Table 1.

No statistically significant differences in the prevalence of depressive symptoms were noted between the two groups of medical students. Overall, 16.6% of respondents had depressive episodes, 16.0% among first-year and 17.9% among sixth-year students. About 9% of all students had thought of committing suicide (Table 2).

Univariate and multivariate logistic regression analyses were used to determine the association of a PHQ-9 score  $\geq 10$  with different risk factors among medical students. Univariate analysis showed a statistically significant association between depressive episodes and self-assessed material status, social health, self-esteem, and use of sleeping pills or sedatives without prescription among first-year students. Among sixth-year students, the association of depressive episodes was significant with social health, self-esteem, and the use of sleeping pills or sedatives without prescription (Table 3). Based on the univariate logistic regression analyses, factors that showed a level of significance of less than 0.05 were selected to be included in the multivariate model. The multivariate logistic regression analysis of PHQ-9 score  $\geq 10$  is presented separately for the first-year students (Table 4) and sixth-year students (Table 5). As shown in Table 4, first-year students with low self-esteem were almost seven times more likely to suffer from moderate to severe depressive symptoms than those with high self-esteem (OR = 6.93, 95% CI: 2.81–17.10). Those who had used sleeping pills or sedatives without a prescription were more than four times more likely to have a PHQ-9 score  $\geq 10$  than those who had not (OR = 4.97, 95% CI: 1.83–13.52). Sixth-year students with self-assessed social relationships as poor or average had increased odds of having a PHQ-9 score  $\geq 10$  (OR = 5.34, 95% CI: 1.26–22.40). Compared with those with high self-esteem, those with low self-esteem were more likely to suffer from moderate to severe depressive symptoms (OR = 10.12, 95% CI: 2.46–41.60) (Table 5).

**Table 1****The demographic and socioeconomic characteristics of medical students in the 1st and 6th year of medical studies**

Characteristics	Year of study			$\chi^2$	p-value
	Total n (%)	1st n (%)	6th n (%)		
Gender					
male	100 (32.5)	64 (30.0)	36 (37.9)	1.845	0.174
female	208 (67.5)	149 (70.0)	59 (62.1)		
Age (years), mean $\pm$ SD	20.95 (2.82)	19.23 (0.88)	24.79 (1.66)		
Self-assessed material status					
low	12 (3.9)	3 (1.4)	9 (9.5)	11.525	<b>0.003</b>
middle	156 (50.6)	112 (52.69)	44 (46.3)		
high	140 (45.5)	98 (46.0)	42 (44.2)		
Self-assessed social health (social relationships)					
poor	13 (4.2)	9 (4.2)	4 (4.2)	1.745	0.418
average	110 (35.7)	71 (33.3)	39 (41.1)		
good	185 (60.1)	133 (62.4)	52 (54.7)		
Self-esteem scale (RSES)					
low self-esteem < 30	82 (26.6)	49 (23.0)	33 (34.7)	4.629	<b>0.031</b>
high self-esteem $\geq$ 30	226 (73.4)	164 (77.0)	62 (65.3)		
Visit to the psychiatrist/psychologist in the last 12 months					
never	277 (89.9)	195 (91.5)	82 (86.3)	2.246	0.325
1–5 times	25 (8.1)	14 (6.6)	11 (11.6)		
> 5 times	6 (1.9)	4 (1.9)	2 (2.1)		
Alcohol use					
2 or more times per week	23 (7.5)	14 (6.6)	9 (9.5)	8.985	<b>0.029</b>
2–4 times per month	121 (39.3)	76 (35.7)	45 (47.4)		
once per month	121 (39.3)	86 (40.4)	35 (36.8)		
never	43 (13.9)	37 (17.4)	6 (6.3)		
Binge drinking					
1 or more times per week	9 (2.9)	6 (2.8)	3 (3.2)	8.925	<b>0.030</b>
once per month	34 (11.0)	24 (11.3)	10 (10.5)		
less than 1 time per month	116 (37.7)	69 (32.4)	47 (49.5)		
never	149 (48.4)	114 (53.5)	35 (36.8)		
Smoking					
current tobacco smoker	60 (19.5)	34 (16.0)	26 (27.4)	7.418	<b>0.025</b>
ever tobacco smoker	31 (10.1)	19 (8.9)	12 (12.6)		
never tobacco smoker	217 (70.5)	160 (75.1)	57 (60.0)		
Lifetime marijuana use					
yes	70 (22.7)	32 (15.0)	38 (40.0)	23.337	<b>&lt; 0.001</b>
no	238 (77.3)	181 (85.0)	57 (60.0)		
Lifetime ecstasy use					
yes	15 (4.9)	8 (3.8)	7 (7.4)	1.851	0.174
no	293 (95.1)	205 (96.2)	88 (92.6)		
Lifetime use of sleeping pills/sedatives without a prescription					
yes	60 (19.5)	30 (14.1)	30 (31.6)	12.819	<b>&lt; 0.001</b>
no	248 (80.5)	183 (85.9)	65 (68.4)		

SD – standard deviation; RSES – Rosenberg self-esteem scale.

Bolded values are statistically significant.

**Table 2****Prevalence of depressive symptoms and suicidal ideation among medical students in the 1st and 6th year**

Parameter	Year of study			$\chi^2$	p-value
	Total (n = 308) n (%)	1st (n = 213) n (%)	6th (n = 95) n (%)		
PHQ-9 score					
0–4 (no significant depressive symptoms)	139 (45.1)	102 (47.9)	37 (38.9)	2.154	0.341
5–9 (mild depressive symptoms)	118 (38.3)	77 (36.1)	41 (43.2)		
$\geq$ 10 (depressive episode)	51 (16.6)	34 (16.0)	17 (17.9)		
Suicidal ideation in the past 2 weeks	27 (8.8)	17 (8.0)	10 (10.5)	0.532	0.466

PHQ-9 – patient health questionnaire-9.

**Table 3****Association of PHQ-9 score  $\geq 10$  with potential risk factors among medical students in the 1st and 6th year**

Risk factors	Univariate logistic regression			
	1st year		6th year	
	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value
Gender				
male	1.00*		1.00*	
female	1.23 (0.54–2.81)	0.620	0.47 (0.16–1.36)	0.164
Self-assessed material status				
high	1.00*		1.00*	
low/middle	2.32 (1.04–5.13)	<b>0.038</b>	0.49 (0.17–1.41)	0.186
Self-assessed social health (social relationships)				
good	1.00*		1.00*	
poor/average	3.82 (1.77–8.26)	<b>0.001</b>	7.88(2.09– 9.78)	<b>0.002</b>
Self-esteem scale (RSES)				
high self-esteem $\geq 30$	1.00*		1.00*	
low self-esteem $< 30$	7.39 (3.35–16.29)	<b>&lt; 0.001</b>	14.49(3.76–55.89)	<b>&lt; 0.001</b>
Alcohol use				
never	1.00*		1.00*	
1 time per month	0.63 (0.22–1.77)	0.381	1.03 (0.10–10.53)	0.977
2–4 times per month	0.88 (0.32–2.44)	0.813	1.25 (0.13–12.07)	0.847
2 or more times per week	1.17 (0.26–5.34)	0.840	0.62 (0.03–12.41)	0.758
Binge drinking				
never	1.00*		1.00*	
less than 1 time per month	0.89 (0.40–1.99)	0.780	3.26 (0.83–12.73)	0.089
1 time per month	0.43 (0.09–1.96)	0.275	2.67 (0.38–18.74)	0.324
1 or more times per week	0.94 (0.10–8.49)	0.956	5.33 (0.37–77.50)	0.220
Smoking				
never tobacco smoker	1.00*		1.00*	
ever tobacco smoker	1.06 (0.29–3.93)	0.928	1.57 (0.36–6.84)	0.551
current tobacco smoker	1.47 (0.57–3.75)	0.421	0.85 (0.24–3.03)	0.808
Lifetime marijuana use				
no	1.00*		1.00*	
yes	1.99 (0.29–3.87)	0.135	1.42 (0.49–4.09)	0.513
Lifetime ecstasy use				
no	1.00*		1.00*	
yes	1.80 (0.35–9.33)	0.483	0.75 (0.08–6.67)	0.796
Lifetime use of sleeping pills/sedatives without a prescription				
no	1.00*		1.00*	
yes	4.03 (1.70–9.53)	<b>0.002</b>	3.05 (1.01–8.95)	<b>0.042</b>

PHQ-9 – patient health questionnaire-9; RSES – Rosenberg self-esteem scale; \* – referent value; OR – odds ratio; CI – confidence interval.

**Bolded values are statistically significant.**

**Table 4****Association of PHQ-9 score  $\geq 10$  with potential risk factors among medical students in the 1st year**

Risk factors	Multivariate logistic regression	
	OR (95% CI)	<i>p</i> -value
Self-assessed material status		
high	1.00*	
low/middle	1.04 (0.43–2.69)	0.934
Self-assessed social health (social relationships)		
good	1.00*	
poor/average	2.26 (0.93–5.46)	0.071
Self-esteem scale (RSES)		
high self-esteem $\geq 30$	1.00*	
low self-esteem $< 30$	6.93 (2.81–17.10)	<b>&lt; 0.001</b>
Lifetime use of sleeping pills/sedatives without a prescription		
no	1.00*	
yes	4.97 (1.83–13.52)	<b>0.002</b>

PHQ-9 – patient health questionnaire-9; RSES – Rosenberg self-esteem scale; \* – referent value; OR – odds ratio; CI – confidence interval.

**Bolded values are statistically significant.**

**Table 5****Association of PHQ-9 score  $\geq 10$  with potential risk factors among medical students in the 6th year**

Risk factors	Multivariate logistic regression	
	OR (95% CI)	<i>p</i> -value
Self-assessed social health		
good	1.00*	
poor/average	5.34 (1.26–22.40)	<b>0.022</b>
Self-esteem scale (RSES)		
high self-esteem $\geq 30$	1.00*	
low self-esteem $< 30$	10.12 (2.46–41.60)	<b>0.001</b>
Sleeping pills/sedatives used without a prescription		
no	1.00*	
yes	1.84 (0.51–6.70)	0.352

**PHQ-9 – patient health questionnaire-9; RSES – Rosenberg self-esteem scale; \* – referent value; OR – odds ratio; CI – confidence interval. Bolded values are statistically significant.**

**Discussion**

This study investigated the prevalence of depressive symptoms and their association with various risk factors in medical students. Forty years ago, a study conducted in 1979 showed that the prevalence rates of all mental disorders among medical students were 16.1% one month after enrolment and 17.5% two years after the first examination<sup>32</sup>. Now, only for depressive symptoms (moderate, moderately severe, and severe), the prevalence was 16.0% in first-year and 17.9% in sixth-year students. However, the prevalence of moderate to severe depressive symptoms in our study was lower than those reported in the previous studies. A recent overview of the literature reported that the prevalence of depressive symptoms among medical students varied across continents, ranging from the lowest in the Western Pacific Region (18.9%) to the highest in Africa (40.9%), and among European medical students was 20.1%<sup>33</sup>. There is significant variation in the prevalence of depressive symptoms between various countries. A very high prevalence of depressive symptoms (60.2%) was found among medical and nursing students in Croatia<sup>34</sup>. The mean prevalence of depression among medical students in China was 32.74%<sup>35</sup>, while in India, it was even higher – 48.4% of the medical students had depressive symptoms<sup>36</sup>. Depressive symptoms prevalence among Italian medical students was 29.5%<sup>37</sup>, which is higher than that among German medical students (20.7%)<sup>38</sup>. Differences in prevalence could be explained by the socio-demographic and cultural differences and the use of different instruments to measure depressive symptoms.

In this study, the prevalence of moderate to severe depressive symptoms among medical students was significantly higher than reported in the general population aged 15–39 years in Vojvodina (1.7%)<sup>39</sup>. The other studies confirmed a higher prevalence of depression in students in clinical and preclinical training groups than in the general public<sup>17, 40–42</sup>. It was also confirmed that the prevalence of depression among medical students is higher than among other students<sup>43</sup>.

The difference in the prevalence of depressive episodes between the first and sixth-year medical students in our study was not statistically significant. In line with our findings, the difference was not found in other research<sup>44, 45</sup>. However, Silva et al.<sup>46</sup> reported that depression scores decreased during medical school, while the other literature suggests that depression worsens with academic training and that prevalence was higher in clinical students than pre-clinical students<sup>47</sup>. We did not find any association between a PHQ-9 score  $\geq 10$  and gender, supporting the findings from meta-analysis<sup>48</sup>. In contrast to our results, literature has shown that female students report more depressive symptoms than males<sup>49–51</sup>.

The previous study conducted among medical students in Serbia showed that 23% of respondents had some suicidal thoughts throughout their lifetime, even only passing ideas<sup>19</sup>. In our study, the prevalence of suicidal ideation in the past two weeks was 8.8%, and it was lower than the prevalence of recent suicidal ideation among medical students in Spain (15.8%)<sup>49</sup> and Germany (14.7%)<sup>38</sup> but higher than in Brazil (7.2%)<sup>52</sup>, China (7.5%)<sup>53</sup>, and the United States (5.7%)<sup>54</sup>.

The worrying fact is that a previous study indicated that most students with high depression scores or thoughts about suicide did not report a current or past diagnosis or treatment of depression<sup>55</sup>. Unfortunately, depression, anxiety, and stress among medical students are often unrecognized and untreated<sup>56, 57</sup>. Furthermore, the prevalence of substance use is quite alarming. Within this sample of sixth-year medical students, 63.2% acknowledged binge drinking, and 40% reported a lifetime history of marijuana use. First-year students who had used sleeping pills or sedatives without a prescription were more likely to have moderate to severe depressive symptoms. The negative impact of mental health problems on students continues after graduation. The existence of a mental disorder may lead to a risk for patients during medical school and even more when the student graduates and enters his or her chosen profession<sup>21</sup>. A review of the literature showed that it adversely affects the quality of patient care, patient safety, and professionalism<sup>18</sup>.

Zeng et al.<sup>45</sup>, in their meta-analysis, argued that excessive academic pressure is a major cause of suicidal ideation and is closely related to other mental health disorders, such as depression. We analyzed different depression-related individual factors. The students in their first year of study who assessed their material status as low or middle had more than two times higher odds for depression than one who was considered high. However, material status was not found to be significantly associated with a PHQ-9 score  $\geq 10$  in multivariable models. In the literature, authors find that socioeconomic factors and medical student characteristics such as low monthly income per capita and low socioeconomic standard were associated with a higher prevalence of depressive symptoms<sup>58, 59</sup>. Moreover, Pham et al.<sup>42</sup> reported that perceived financial burden was found to be a significant factor associated with self-reported depression.

Final-year medical students with poor or average social relationships had more than five times higher odds of having moderate to severe depressive symptoms. Association was not found to be significant among first-year students. A possible explanation for such results could be that sixth-year students, due to excessive academic overload, have lost their social ties resulting in poor social relationships, and do not use this crucial resource for mental well-being. Increased satisfaction with social activities is a well-known protective factor for depression in medical students<sup>46</sup>. Mahroon et al.<sup>60</sup> found a statistically significant relationship between the quality of relationships with peers and the prevalence of depressive symptoms.

Multivariate analysis showed a significant association between low self-esteem and a PHQ-9 score  $\geq 10$  in both first and final-year students. Self-esteem refers to a person's positive or negative attitude toward themselves<sup>28</sup>. The literature demonstrates that adolescents with high self-esteem suffer fewer symptoms of depression over time<sup>61</sup>. As implied by Mann et al.<sup>62</sup>, it is a bidirectional process. A pessimistic view of themselves can lead to depressive feelings, but de-

pression or lack of efficient functioning could lead to feeling bad, which might decrease self-esteem. High self-esteem can act as a resilience factor against depression<sup>28</sup> because high self-esteem people appear to use better self-regulation strategies than low self-esteem people<sup>63</sup>.

#### *Limitations of the study*

This study has several limitations. All responses were self-reported, making our results susceptible to recall bias. Additionally, other variables, such as previous psychiatric illnesses that might influence the findings, were not included. The limitations include the cross-sectional structure, making it impossible to draw any conclusions about cause and effect.

#### **Conclusion**

There is a high prevalence of depressive episodes among first- and sixth-year medical students in the Medical Faculty of Novi Sad, and it is associated with low self-esteem in both groups, use of sleeping or sedative pills without a prescription among first-year students, and poor social health in sixth-year students. We recommend routine screening for depression in medical students and establishing prevention and intervention programs.

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#### **Conflict of interest**

The authors declare no conflict of interest.

#### R E F E R E N C E S

1. Patton GC, Sawyer SM, Santelli JS, Ross DA, Afifi R, Allen NB, et al. Our future: a Lancet commission on adolescent health and wellbeing. *Lancet* 2016; 387(10036): 2423–78.
2. Patton GC, Coffey C, Romaniuk H, Mackinnon A, Carlin JB, Degenhardt L, et al. The prognosis of common mental disorders in adolescents: a 14-year prospective cohort study. *Lancet* 2014; 383(9926): 1404–11.
3. Duffy A, Saunders KEA, Malhi GS, Patten S, Cipriani A, McNeven SH, et al. Mental health care for university students: a way forward? *Lancet Psychiatry* 2019; 6(11): 885–87.
4. Yusoff MSB, Mat Pa MN, Esa AR, Abdul Rahim AF. Mental health of medical students before and during medical education: A prospective study. *J Taibah Univ Med Sci* 2013; 8(2): 86–92.
5. Smith CK, Peterson DF, Degenhardt BF, Johnson JC. Depression, anxiety, and perceived hassles among entering medical students. *Psychol Health Med* 2007; 12(1): 31–9.
6. AlFaris E, Irjan F, Qureshi R, Naem N, Alsbomrani A, Ponnampetrum G, et al. Health professions' students have an alarming prevalence of depressive symptoms: exploration of the associated factors. *BMC Med Educ* 2016; 16(1): 279.
7. Heinen I, Bullinger M, Kocalevent RD. Perceived stress in first year medical students - associations with personal resources and emotional distress. *BMC Med Educ* 2017; 17(1): 4.
8. Gan GG, Yuen Ling H. Anxiety, depression and quality of life of medical students in Malaysia. *Med J Malaysia* 2019; 74(1): 57–61.
9. Pagnin D, de Queiroz V. Comparison of quality of life between medical students and young general populations. *Educ Health (Abingdon)* 2015; 28(3): 209–12.
10. Hill MR, Goicochea S, Merlo LJ. In their own words: stressors facing medical students in the millennial generation. *Med Educ Online* 2018; 23(1): 1530558.
11. Oura MJ, Moreira AR, Santos P. Stress among Portuguese Medical Students: A National Cross-Sectional Study. *J Environ Public Health* 2020; 2020: 6183757
12. Liranso GS, Mohan SS, Prakash M, Vicky VD. Mental Health Distress and Wellness among Medical Students. *J Neurol Neurol Disord* 2018; 4(1): 102.
13. Cuttilan AN, Sayampathan AA, Ho RC. Mental health issues amongst medical students in Asia: a systematic review [2000–2015]. *Ann Transl Med* 2016; 4(4): 72.



14. Pacheco JP, Giacomini HT, Tam WW, Ribeiro TB, Arab C, Bezerra IM, et al. Mental health problems among medical students in Brazil: a systematic review and meta-analysis. *Braz J Psychiatry* 2017; 39(4): 369–78.
15. Lew B, Huen J, Yu P, Yuan L, Wang DF, Ping F, et al. Associations between depression, anxiety, stress, hopelessness, subjective well-being, coping styles and suicide in Chinese university students. *PLoS One* 2019; 14(7): e0217372.
16. Bauer RL, Chesin MS, Jeglic EL. Depression, delinquency, and suicidal behaviors among college students. *Crisis* 2014; 35(1): 36–41.
17. Rotenstein LS, Ramos MA, Torre M, Segal JB, Peluso MJ, Guille C, et al. Prevalence of depression, depressive symptoms, and suicidal ideation among medical students: a systematic review and meta-analysis. *JAMA* 2016; 316(21): 2214–36.
18. Dyrbye LN, Thomas MR, Shanafelt TD. Systematic review of depression, anxiety, and other indicators of psychological distress among U.S. and Canadian medical students. *Acad Med* 2006; 81(4): 354–73.
19. Miletić V, Luković JA, Ratković N, Aleksić D, Grgurević A. Demographic risk factors for suicide and depression among Serbian medical school students. *Soc Psychiatry Psychiatr Epidemiol* 2015; 50(4): 633–8.
20. Ristić-Ignjatović D, Hinić D, Jakovljević M, Fountoulakis K, Siepera M, Rancić N. A ten-year study of depressive symptoms in Serbian medical students. *Acta Clin Croat* 2013; 52(2): 157–63.
21. Royal College of Psychiatrists. Mental health of students in higher education. September, 2011. [cited 2021 Aug 21.] Available from: [https://www.rcpsych.ac.uk/docs/defaultsource/improving-care/better-mh-policy/college-reports/college-reportcr166.pdf?sfvrsn=d5fa2c24\\_2](https://www.rcpsych.ac.uk/docs/defaultsource/improving-care/better-mh-policy/college-reports/college-reportcr166.pdf?sfvrsn=d5fa2c24_2)
22. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: Validity of a Brief Depression Severity Measure. *J Gen Intern Med* 2001; 16(9): 606–13.
23. Rosenberg M. Society and the adolescent self-image. Princeton, NJ: Princeton University Press; 1965.
24. Levis B, Benedetti A, Thombs BD. Accuracy of Patient Health Questionnaire-9 (PHQ-9) for screening to detect major depression: individual participant data meta-analysis. *BMJ* 2019; 365: 11476.
25. Ademuyi AO, Ola BA, Afolabi OO. Validity of the Patient Health Questionnaire (PHQ-9) as a Screening Tool for Depression Amongst Nigerian University Students. *J Affect Disord* 2006; 96(1–2): 89–93.
26. Kim YE, Lee B. The Psychometric Properties of the Patient Health Questionnaire-9 in a Sample of Korean University Students. *Psychiatry Investig* 2019; 16(12): 904–10.
27. Bauer AM, Chan YF, Huang H, Vannoy S, Uniützer J. Characteristics, management, and depression outcomes of primary care patients who endorse thoughts of death or suicide on the PHQ-9. *J Gen Intern Med* 2013; 28(3): 363–9.
28. Simon GE, Rutter CM, Peterson D, Oliver M, Whiteside U, Operskalski B, et al. Does response on the PHQ-9 Depression Questionnaire predict subsequent suicide attempt or suicide death? *Psychiatr Serv* 2013; 64(12): 1195–202.
29. Saleh D, Camart N, Romo L. Predictors of Stress in College Students. *Front Psychol* 2017; 8: 19.
30. Tagarroa M, Galinha S. Adaptation of Rosenberg's Self-esteem scale and EBEPs-A Self-esteem Subscale on Portuguese students. *Eur J Behav Sci* 2017; 17(3): 188–97.
31. Desai A. Governments confront drunken violence. *Bull World Health Organ* 2010; 88(9): 644–5.
32. Erić L, Radovanović Z, Jevremović I. Mental disorders among Yugoslav medical students. *Br J Psychiatry* 1988; 152: 127–9.
33. Tam W, Lo K, Pacheco J. Prevalence of depressive symptoms among medical students: overview of systematic reviews. *Med Educ* 2019; 53(4): 345–54.
34. Milić J, Škrlec I, Milić Vranješ I, Podgornjak M, Heffer M. High levels of depression and anxiety among Croatian medical and nursing students and the correlation between subjective happiness and personality traits. *Int Rev Psychiatry* 2019; 31(7–8): 653–60.
35. Mao Y, Zhang N, Liu J, Zhu B, He R, Wang X. A systematic review of depression and anxiety in medical students in China. *BMC Med Educ* 2019; 19(1): 327.
36. Kumar SG, Kattimani S, Sarkar S, Kar SS. Prevalence of depression and its relation to stress level among medical students in Puducherry, India. *Ind Psychiatry J* 2017; 26(1): 86–90.
37. Bert F, Lo Moro G, Corradi A, Acampora A, Agodi A, Brunelli L, et al.; Collaborating Group. Prevalence of depressive symptoms among Italian medical students: The multicentre cross-sectional "PRIMES" study. *PLoS One* 2020; 15(4): e0231845.
38. Chow WS, Schmidtke J, Loerbroks A, Muth T, Angerer P. The Relationship between Personality Traits with Depressive Symptoms and Suicidal Ideation among Medical Students: A Cross-Sectional Study at One Medical School in Germany. *Int J Environ Res Public Health* 2018; 15(7): 1462.
39. Harbaji S. Socio-medical aspects of depressive disorders in Vojvodina [dissertation]. Novi Sad: Faculty of Medicine, University of Novi Sad; 2016. (Serbian)
40. Steiner-Hofbauer V, Holzinger A. How to Cope with the Challenges of Medical Education? Stress, Depression, and Coping in Undergraduate Medical Students. *Acad Psychiatry* 2020; 44(4): 380–7.
41. Ediz B, Ozcakir, A, Bilgel, N. Depression and anxiety among medical students: Examining scores of the Beck depression and anxiety inventory and the depression anxiety and stress scale with student characteristics. *Cogent Psychology* 2017; 4(1): 283829.
42. Pham T, Bui L, Nguyen A, Nguyen B, Tran P, Vu P, et al. The prevalence of depression and associated risk factors among medical students: An untold story in Vietnam. *PLoS One* 2019; 14(8): e0221432.
43. Lei XY, Xiao LM, Liu YN, Li YM. Prevalence of Depression among Chinese University Students: A Meta-Analysis. *PLoS One* 2016; 11(4): e0153454.
44. Bassols AM, Okabayashi LS, Silva AB, Carneiro BB, Feijó F, Guimarães GC, et al. First- and last-year medical students: is there a difference in the prevalence and intensity of anxiety and depressive symptoms? *Braz J Psychiatry* 2014; 36(3): 233–40.
45. Zeng W, Chen R, Wang X, Zhang Q, Deng W. Prevalence of mental health problems among medical students in China: A meta-analysis. *Medicine (Baltimore)* 2019; 98(18): e15337.
46. Silva V, Costa P, Pereira I, Faria R, Salgueira AP, Costa MJ, et al. Depression in medical students: insights from a longitudinal study. *BMC Med Educ* 2017; 17(1): 184.
47. Ngasa SN, Sama CB, Dzekem BS, Nforchu KN, Tindong M, Aroke D, et al. Prevalence and factors associated with depression among medical students in Cameroon: a cross-sectional study. *BMC Psychiatry* 2017; 17(1): 216.
48. Puthran R, Zhang MW, Tam WW, Ho RC. Prevalence of depression amongst medical students: a meta-analysis. *Med Educ* 2016; 50(4): 456–68.
49. Atienza-Carbonell B, Balanzá-Martínez V. Prevalence of depressive symptoms and suicidal ideation among Spanish medical students. *Actas Esp Psiquiatr* 2020; 48(4): 154–62.
50. Brenneisen Mayer F, Souza Santos I, Silveira PS, Itaquí Lopes MH, de Souza AR, Campos EP, et al. Factors associated to depression and anxiety in medical students: a multicenter study. *BMC Med Educ* 2016; 16(1): 282.
51. Burger PHM, Scholz M. Gender as an underestimated factor in mental health of medical students. *Ann Anat* 2018; 218: 1–6.

52. *Torres AR, Campos LM, Lima MCP, Ramos-Cerqueira ATA.* Suicidal Ideation Among Medical Students: Prevalence and Predictors. *J Nerv Ment Dis* 2018; 206(3): 160–8.
53. *Sobonale K, Zhou N, Fan J, Liu N, Sherer R.* Depression and suicidal ideation in medical students in China: a call for wellness curricula. *Int J Med Educ* 2014; 5: 31–6.
54. *Goebert D, Thompson D, Takeshita J, Beach C, Bryson P, Ephgrave K, et al.* Depressive symptoms in medical students and residents: a multischool study. *Acad Med* 2009; 84(2): 236–41.
55. *Schwenk TL, Davis L, Wimsatt LA.* Depression, stigma, and suicidal ideation in medical students. *JAMA* 2010; 304(11): 1181–90.
56. *Menon V, Sarkar S, Kumar S.* Barriers to healthcare seeking among medical students: A cross sectional study from South India. *Postgrad Med J* 2015; 91(1079): 477–82.
57. *Thompson G, McBride RB, Hosford CC, Halaas G.* Resilience Among Medical Students: The Role of Coping Style and Social Support. *Teach Learn Med* 2016; 28(2): 174–82.
58. *Pan XF, Wen Y, Zhao Y, Hu JM, Li SQ, Zhang SK, et al.* Prevalence of depressive symptoms and its correlates among medical students in China: a national survey in 33 universities. *Psychol Health Med* 2016; 21(7): 882–9.
59. *Abdel Wabed WY, Hassan SK.* Prevalence and associated factors of stress, anxiety and depression among medical Fayoum University students. *Alexandria J Med* 2017; 53(1): 77–84.
60. *Mabroon ZA, Borgan SM, Kamel C, Maddison W, Royston M, Donnellan C.* Factors Associated with Depression and Anxiety Symptoms Among Medical Students in Bahrain. *Acad Psychiatry* 2018; 42(1): 31–40.
61. *Henriksen IO, Ranøyen I, Indredavik MS, Stenseng F.* The role of self-esteem in the development of psychiatric problems: a three-year prospective study in a clinical sample of adolescents. *Child Adolesc Psychiatry Ment Health* 2017; 11: 68.
62. *Mann M, Hosman CM, Schaalma HP, de Vries NK.* Self-esteem in a broad-spectrum approach for mental health promotion. *Health Educ Res* 2004; 19(4): 357–72.
63. *Baumeister RF, Campbell JD, Krueger JI, Vohs KD.* Does High Self-Esteem Cause Better Performance, Interpersonal Success, Happiness, or Healthier Lifestyles? *Psychol Sci Public Interest* 2003; 4(1): 1–44.

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# An economic evaluation of phacoemulsification and extracapsular cataract extraction in cataract surgery

## Farmakoekonomska evaluacija fakoemulzifikacije i ekstrakapsularne ekstrakcije u operaciji katarakte

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### Abstract

**Background/Aim.** Cataract surgery is one of the most often performed surgical interventions. The predominant method in Western countries is phacoemulsification, while in developing countries, the extracapsular cataract extraction (ECCE) method remains popular. The aim of the study was to evaluate the cost-effectiveness of these two cataract surgery techniques from the provider's perspective if operation complications were the outcome of the interest. **Methods.** The data were obtained from the Department of Ophthalmology of the General Hospital Kruševac during a one-year period. A total of 1,179 surgeries by five surgeons were performed. The cost-effectiveness was evaluated using the decision tree. All probabilities were calculated based on the likelihood of the occurrence during the study period. Only direct costs were considered, and values were taken from the documentation at the hospital and the official price list of health services. One- and two-way sensitivity

analyses were performed. **Results.** The total cost per patient in the phacoemulsification group was 71,008.70 Serbian dinars (RSD), while the total cost in the ECCE group was 74,340.36 RSD. At the same time, phacoemulsification shows higher effectiveness than the ECCE method, with 87% and 57% of patients without complications, respectively. With these results, phacoemulsification was the dominant strategy compared to ECCE. The sensitivity analysis revealed that the results are sensitive to the number of performed operations per year. **Conclusion.** The phacoemulsification technique seems to be the preferred technique for cataract surgery. All the investment in phacoemulsification equipment and consumables is justified if the number of surgeries per year exceeds 350.

### Key words:

cataract; cost-benefit analysis; ophthalmologic surgical procedures; phacoemulsification; economics, pharmaceutical; serbia.

### Apstrakt

**Uvod/Cilj.** Operacija katarakte predstavlja jednu od najčešće primenjivanih hirurških intervencija. U zapadnim zemljama, dominantna tehnika je fakoemulzifikacija, dok je u zemljama u razvoju najzastupljenija tehnika ekstrakapsularne ekstrakcije (ECCE). Cilj rada bio je da se proceni ekonomska isplativost te dve tehnike operacije katarakte iz perspektive pružaoca usluge, ukoliko se kao ishod posmatraju komplikacije. **Metode.** Podaci su dobijeni sa Očnog odeljenja Opšte bolnice Kruševac tokom jednogodišnjeg perioda. Ukupno je izvedeno 1 179 operacija od strane pet hirurga. Ekonomska isplativost je procenjena primenom „drveta odlučivanja“. Verovatnoće za događaje su izračunate na osnovu verovatnoće pojavljivanja tokom navedenog perioda. U analizi su razmatrani samo direktni troškovi, a vrednosti su preuzete iz prateće dokumentacije i zvaničnog cenovnika zdravstvenih usluga. Sprovedena je jednosmerna i dvosmerna analiza osetljivosti. **Rezultati.**

Ukupni troškovi u grupi koja je bila podvrgnuta fakoemulzifikaciji iznosili su 71 008.70 srpskih dinara (RSD), dok su u ECCE grupi oni iznosili 74 340.36 RSD. Istovremeno, fakoemulzifikacija je pokazala višu efikasnost u odnosu na ECCE, 87% i 57% bolesnika bez komplikacija, redom. Na osnovu dobijenih rezultata, fakoemulzifikacija je bila dominantna strategija u poređenju sa ECCE. Analiza osetljivosti pokazala je da su rezultati osetljivi na broj izvršenih intervencija na godišnjem nivou. **Zaključak.** Fakoemulzifikacija je ekonomski isplativija tehnika operacije katarakte u odnosu na ECCE. Sva ulaganje u opremu i potrošni materijal za fakoemulzifikaciju opravdani su ukoliko je broj izvedenih operacija na godišnjem nivou preko 350.

### Ključne reči:

katarakta; troškovi-korist, analiza; hirurgija, oftalmološka, procedure; fakoemulzifikacija; farmakoekonomika; srbija.

## Introduction

Visual impairment is a significant problem both for the affected individual and society in economic and social terms. According to the latest data from the World Health Organization, it is estimated that 39 million people worldwide are blind. The most significant cause of preventable blindness is cataract<sup>1,2</sup>.

Prevalence rates of cataracts and blindness globally depend on the examination method, such as Lens Opacities Classification System (LOCS) III or Optical Quality Analysis System (OQAS), and diagnostic cut-off value used for best corrected visual acuity (VA) or presenting VA. However, the risk of cataracts increases with each decade of life, starting around age 40. Half of white Americans had cataracts by 75 years of age in 2010. It is forecasted that the number of people with cataracts will double from 24.4 million to about 50 million by the year 2050<sup>3</sup>. Results from Europe vary significantly, depending on the region, population, examination method, etc. The study from 2013 reflects this difference, from 6% in the Netherlands to almost 70% in Spain<sup>4</sup>. The most recent study from Sweden showed that the crude prevalence was 31.5%, with a higher percentage in the male population<sup>5</sup>. In Serbia, cataract was the second most frequent reason for hospitalizations in females in 2017, after breast cancer<sup>6</sup>.

Cataract surgery is one of the most often performed surgical interventions in developed countries. The predominant method of cataract surgery in Western countries is phacoemulsification. In randomized clinical trials, it produces better outcomes than extracapsular cataract extraction (ECCE)<sup>7</sup>. However, the ECCE method remains prevalent in developing countries due to its cost-effectiveness<sup>8</sup>.

The WHO has suggested that an annual rate of 350 surgeries per 100,000 inhabitants is a useful target against cataract blindness<sup>9</sup>.

This study aimed to compare the cost-effectiveness of two cataract surgery techniques, phacoemulsification and ECCE, by considering the occurrence of possible complications of the operation from the perspective of the health insurance (third-party payer).

## Methods

The data for the analysis was obtained from the Department of Ophthalmology of the General Hospital Kruševac, Serbia. The hospital is the only public hospital that provides healthcare services for approximately 260,000 people in the Rasina district.

This study covered one year (from March 2019 to February 2020). During the study period, a total of 1,179 cataract surgeries (1,123 phacoemulsifications and 56 ECCE) were performed by five surgeons. All interventions were included in the analysis. Data on the efficacy of these two methods were calculated from patients' medical records. The retrospective analysis determined the number of patients with complications of cataract surgery that occurred in one or the other surgery method. This study was

approved by the hospital's Ethical Committee (No. 09/21 EO from April 19, 2021).

The cost-effectiveness of two types of cataract surgery was evaluated using the decision tree (Figure 1).

### *Probabilities*

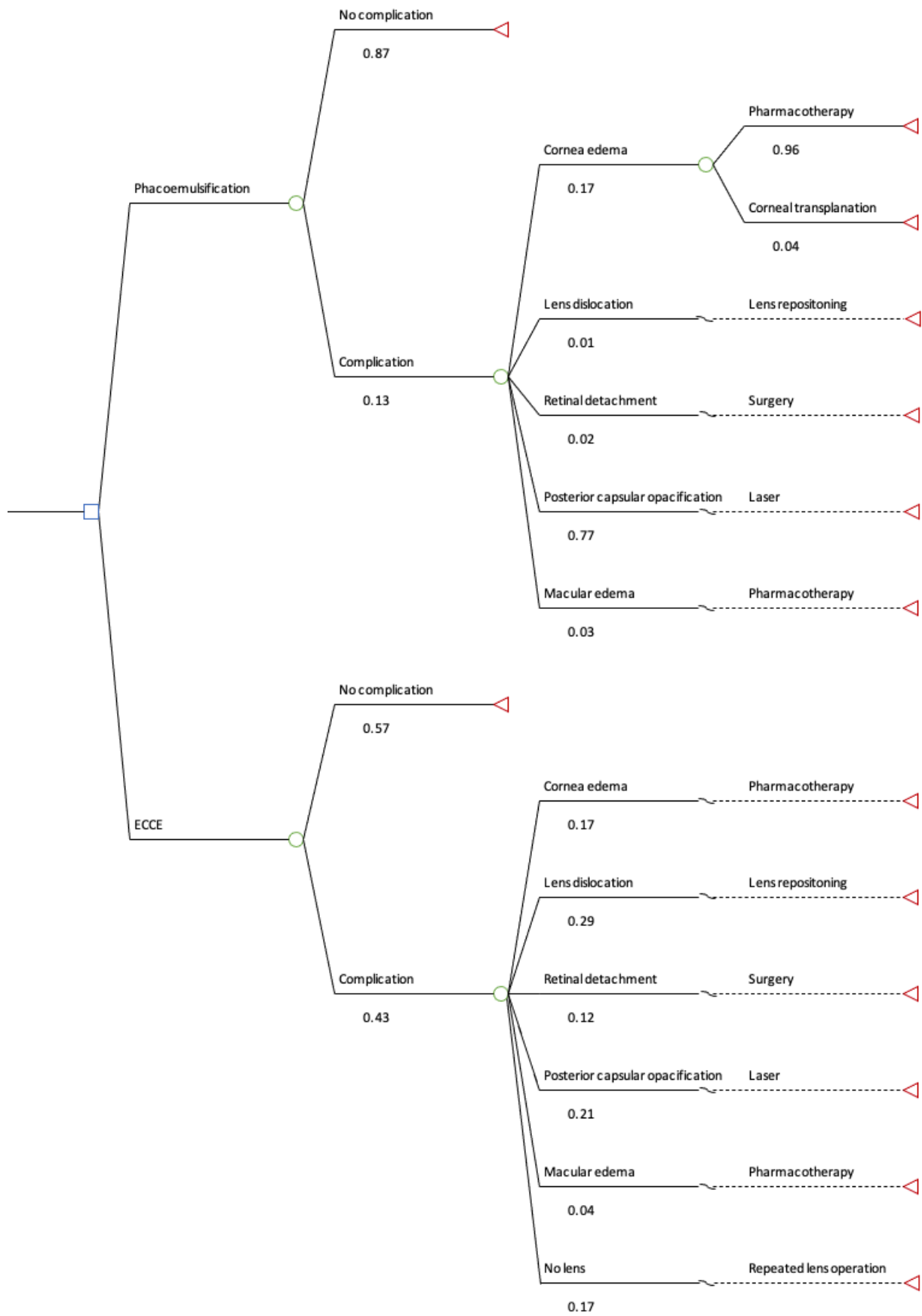
As the analysis outcome, complications during and after surgery were observed. In the phacoemulsification group, 146 out of 1,123 (13%) patients had complications, with 77% of posterior capsule opacification as the main complication. In the ECCE group, 43% of patients had complications, dominantly lens dislocation, occurring in 29% of cases. Probabilities for all outcomes and both techniques of surgery are presented in Figure 1.

### *Costs*

Only direct costs were considered in the analysis. All costs are expressed in Serbian dinars (RSD), for which an exchange rate was as follows: 1 EUR = 117.5736 RSD for the year 2021, according to the National Bank of Serbia. Cost data were taken from the administrative documentation at the hospital and the official price list of health services of the Republic Institute for Health Insurance<sup>10</sup>. In the analysis, the following costs were included: the first consultation with an ophthalmologist, at which the indication for cataract surgery and scheduling of the operation itself happened; admission for surgery (Table 1); costs of the procedure itself (Table 2); follow-up controls after one and three weeks of the surgery; capital cost for the phacoemulsification machine. Besides the two previously mentioned controls, the ECCE group of patients also included the costs for the third and fourth control after surgery necessary for patients operated by this method.

Table 2 shows the costs of medicines, materials, and services provided during the cataract surgery itself. Most consumables are the same; the most prominent differences were for the lenses used – soft with the phacoemulsification method, hard with the ECCE method, and consumables for the phacoemulsification machine. During phacoemulsification, two types of knives are used, while the ECCE method uses one kind of knife, but also surgical suture is necessary afterward. Furthermore, patients in the ECCE group have an additional day of hospitalization compared to the phacoemulsification group.

The capital cost was included in the phacoemulsification group since it represented a fixed expense incurred on the purchase of equipment used for surgery. That consisted of the cost of the phacoemulsification machine itself, machine consumables, service, and instruments, considering that 1,000 operations are performed annually. The price of the phacoemulsification machine was 4.13 million RSD, and it has depreciated over five years. The cost per patient is calculated by dividing the machine's price by the estimated number of operations over five years (5,000). The cost of machine consumables per patient is calculated in the same manner.



**Fig. 1 – Decision tree used in the model.  
ECCE – extracapsular cataract extraction.**

**Table 1****Costs on admission to the hospital prior to surgery included in the analysis (independent of the technique performed).**

Variable	Number of units	Unit cost RSD	Total cost RSD
Specialist consultation	1	186.98	186.98
Topical anesthesia	2	58.30	116.60
Ophthalmoscopy	1	1,340.00	1,340.00
Ocular tonometry	1	1,600.00	1,600.00
Eye irrigation	1	93.49	93.49
Visual acuity	1	1,600.00	1,600.00
Consumables (compress, solutions, etc.)			7.68
<b>Total</b>			<b>4,944.75</b>

**RSD – Serbian dinars.**

**Table 2****Costs of services, medicines, medical devices, and consumables provided during the cataract surgery**

Variable	ECCE		Phacoemulsification	
	number of units	RSD	number of units	RSD
<b>Services</b>				
peribulbar application of an anesthetic surgery	1	1,800.00	1	1,800.00
subconjunctival application of medicine	1	38,284.19	1	38,284.19
sedation	1	1,340.00	1	1,340.00
in-patient day at the general ward	1	2,810.00	1	2,810.00
in-patient day at the semi-intensive unit	1	1,545.40	1	1,545.40
	2	4,467.08	1	2,233.54
<b>Medicines</b>				
Lens		574.63		553.33
Knifes	1	682.00	1	3,626.70
Other consumables	1	422.40	2	730.80
<b>Total</b>		<b>4,599.68</b>		<b>4,524.67</b>
		<b>55,185.37</b>		<b>57,448.62</b>

**ECCE – extracapsular cataract extraction; RSD – Serbian dinars.**

Moreover, the costs of complications are included in the analysis according to the administrative documentation for each patient with complications. The only exception was the cost of medicines used to treat corneal edema and macular edema, considering that the pharmacotherapy is applied at home.

*Analysis*

All calculations and analyses were performed using Microsoft Excel version 16 (Microsoft Corporation, USA, 2019). In cost-effectiveness analysis, results are presented as cost per patient without complications. An incremental cost-effectiveness ratio (ICER) analysis was also performed. To assess the robustness of the model, a one- and two-way sensitivity analysis was performed. The following variables were tested in the sensitivity analysis: the annual number of operations with the phacoemulsification method and the effectiveness of the technique itself.

**Results**

The results of the cost-effectiveness analysis are shown in Table 3. Considering all the costs included in the study, the total cost per patient in the phacoemulsification group was 71,008.70 RSD, while the total cost in the ECCE group was 74,340.36 RSD. Considering the method's effectiveness regarding the percentage of patients without complications, phacoemulsification shows higher effectiveness (87%) than the ECCE method (57%). Due to these findings, cost-effectiveness analysis shows that phacoemulsification is the dominant strategy compared to the ECCE method, meaning a technique with lower costs (-3,331.66 RSD) and higher effectiveness (0.3).

One-way sensitivity analyses were performed for an annual number of operations with the phacoemulsification method. The results were sensitive to this variable, where phacoemulsification stops being the dominant strategy if the number of operations drops below 350 per year. The two-

**Table 3****Results of the cost-effectiveness analysis**

Technique	Effect	Δ effect	Cost (RSD)	Δ cost	ICER
ECCE	0.57		74,340.36		
Phacoemulsification	0.87	0.30	71,008.70	-3,331.66	Dominant (-11,105.53)

**ECCE – extracapsular cataract extraction; ICER – incremental cost-effectiveness ratio; RSD – Serbian dinars.**

way sensitivity analyses (number of operations and effectiveness of the phacoemulsification method) show that results were not sensitive to the effectiveness of the phacoemulsification method.

### Discussion

According to the study results, phacoemulsification was more effective than the ECCE method due to a lower rate of complications (13% vs. 43%). That was similar to the Malaysian and Australian studies, with more common complications with the ECCE than the phacoemulsification technique<sup>11-12</sup>.

On the side of the costs, the results of our study show lower costs associated with phacoemulsification as a possible method of cataract surgery. A combination of higher effectiveness and lower costs results in the dominant status of phacoemulsification. Additionally, according to the analysis, the result is sensitive to the number of operated patients per year. Results from our study are comparable with those from Kara-Junior et al.<sup>13</sup>, which concluded a significant economic advantage in favor of the phacoemulsification technique, especially if the patient is working. A recent observational study from India showed similar results since phacoemulsification was found to be more cost-effective compared to other cataract techniques<sup>14</sup>. The study by Muralikrishnan et al.<sup>15</sup> estimated the cost of different cataract surgery procedures by applying various perspectives. Contrary to our results, the authors revealed that phacoemulsification is associated with the highest costs assuming the same perspective as in our study, due to the need for expensive equipment and consumables. Identical results were obtained in India, where phacoemulsification had higher provider direct costs (25.55 USD) compared with ECCE (16.25 USD), which were attributed to the cost of equipment and consumables<sup>16</sup>.

The first published cost-effectiveness analysis revealed lower costs associated with ECCE compared to the phacoemulsification method in Australia<sup>17</sup>. The main reason for the difference in the results is that the authors assumed no difference between the two procedures based on expert opinion. Additionally, the authors concluded that any increase in the use of the phacoemulsification method results in decreasing costs, which is in line with our results.

Two studies conducted in Malaysia showed that the costs of phacoemulsification were higher than ECCE. A study from 2004 indicated no significant difference in cost-effectiveness between the two methods<sup>18</sup>, while a study from 2007 showed that ECCE is more cost-effective than phacoemulsification<sup>8</sup>. The difference with the results obtained in our study can be explained by the used unit of effectiveness. Namely, the studies used VA or vision-related quality of life evaluated by questionnaire.

The Cochrane Systematic Review from 2014 concluded that phacoemulsification results in better visual outcomes and a lower complication rate compared to ECCE. Still, the lower cost of ECCE may justify its use by maximizing the number of people that can be treated with restrained resources<sup>19</sup>.

Evidence from different settings suggests that cataract surgery is cost-effective in developing countries and Western countries, with the range of results from cost-utility analysis from 9 USD to 25,000 USD per quality-adjusted life year<sup>20-23</sup>. Similar results were obtained using the other type of outcome, disability-adjusted life year<sup>24</sup>.

The results of our analysis support the use of phacoemulsification as a dominant surgical method in General Hospital Kruševac. The study results from Poland also revealed phacoemulsification as the preferred surgical technique for cataracts<sup>25</sup>.

Although the pharmacoeconomic evaluation was introduced more than one decade ago into the Serbian health care system, its influence is humble and rather advisory, especially in other health technologies besides medicines, such as surgical or diagnostic procedures.

This economic evaluation has some limitations. The sample for the study was taken retrospectively, and it reflects current clinical practice with a disproportional number of techniques. Moreover, the analysis did not include the time needed for the surgeon phacoemulsification training program since all surgeons included in the analysis are senior, experienced surgeons. The inclusion of this variable would alter the results and conclusion since published studies show a high learning curve<sup>26</sup>. The authors desired to perform a cost-utility analysis, but due to the unavailability of the patients, it was not possible to apply quality of life questionnaire to include humanistic outcomes in the study.

### Conclusion

The phacoemulsification technique seems to be the preferred technique for cataract surgery based on the results of the pharmacoeconomic analysis. The study results justify the investment for phacoemulsification equipment and consumables if the number of surgeries per year in the health care institution is above 350. The phacoemulsification method in a healthcare institution is linked with clear economic and clinical outcomes, such as savings in monetary resources and a lower rate of complications.

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## R E F E R E N C E S

- Nikolić Lj. Cataract surgery. 1<sup>st</sup> ed. Belgrade: Zavod za udžbenike; 2009. (Serbian)
- WHO. Blindness and vision impairment. Available from: <https://www.who.int/en/news-room/fact-sheets/detail/blindness-and-visual-impairment> [accessed 2021 April 21].
- National Eye Institute. Cataract Data and Statistics. Available from: <https://www.nei.nih.gov/learn-about-eye-health/resources-for-health-educators/eye-health-data-and-statistics/cataract-data-and-statistics> [accessed 2021 April 21].
- Prokofjeva E, Wegener A, Zrenner E. Cataract prevalence and prevention in Europe: a literature review. *Acta Ophthalmol* 2013; 91(5): 395–405.
- Hugosson M, Ekström C. Prevalence and risk factors for age-related cataract in Sweden. *Ups J Med Sci* 2020; 125(4): 311–5.
- Institute of Public Health "Dr. Milan Jovanovic Batut". Selected health indicators for 2017. Belgrade: Institute of Public Health "Dr. Milan Jovanovic Batut"; 2018. (Serbian)
- American Academy of Ophthalmology. Cataract in the Adult Eye Preferred Practice Pattern 2016. San Francisco, CA: American Academy of Ophthalmology; 2016.
- Manaf MRA, Aljunid SM, Annuar FH, Leong CK, Mansor N. Cost-effectiveness analysis of cataract surgery with intraocular lens implantation: extracapsular cataract extraction versus phacoemulsification. *Med J Indones* 2007; 16(1): 25–31.
- Assia-Pacific Association of Cataract & Refractive Surgeons (APACRS). Principles of Preferred Practice in Cataract Surgery. Available from: [https://apacrs.org/ppp\\_cataract\\_EN/index.html#6](https://apacrs.org/ppp_cataract_EN/index.html#6)
- Republic Health Insurance Fund. Rulebook on prices of health services at the secondary and tertiary level. ("Sl. glasnik RS", br. 55/2019). (Serbian)
- Thevi T, Reddy SC, Shantakumar C. Outcome of phacoemulsification and extracapsular cataract extraction: A study in a district hospital in Malaysia. *Malays Farm Physician* 2014; 9(2): 41–7.
- Clark A, Morlet N, Ng JQ, Preen DB, Semmens JB. Whole population trends in cataract surgery over 22 years in Western Australia. *Ophthalmology* 2011; 118(6): 1055–60.
- Kara N Jr, Sirtoli MG, Santhiago MR, Parede TR, Espíndola RF, Carvalho Rde S. Phacoemulsification versus extracapsular extraction: governmental costs. *Clinics (Sao Paulo)* 2010; 65(4): 357–61.
- Rochmab TN, Wulandari A, Dablui M, Ernawaty, Wulandari RD. Cost Effectiveness Analysis Using Disability-Adjusted Life Years for Cataract Surgery. *Int J Environ Res Public Health* 2020; 17(16): 6010.
- Muralikrishnan R, Venkatesh R, Prajna NV, Frick KD. Economic cost of cataract surgery procedures in an established eye care centre in Southern India. *Ophthalmic Epidemiol* 2004; 11(5): 369–80.
- Agarwal A. Measuring the cost-effectiveness of cataract surgery. Available from: <https://www.healio.com/news/ophthalmology/20120325/measuring-the-cost-effectiveness-of-cataract-surgery> [accessed 2022 February 03].
- Asimakis P, Coster DJ, Lewis DJ. Cost effectiveness of cataract surgery. A comparison of conventional extracapsular surgery and phacoemulsification at Flinders Medical Centre. *Aust N Z J Ophthalmol* 1996; 24(4): 319–25.
- Loo CY, Kandiah M, Arumugam G, Gob PP, John E, Gurusami B, et al. Cost efficiency and cost effectiveness of cataract surgery at the Malaysian Ministry of Health ophthalmic services. *Int Ophthalmol* 2004; 25(2): 81–7.
- de Silva SR, Riaz Y, Evans JR. Phacoemulsification with posterior chamber intraocular lens versus extracapsular cataract extraction (ECCE) with posterior chamber intraocular lens for age-related cataract. *Cochrane Database Syst Rev* 2014; (1): CD008812.
- Griffiths UK, Bozzani FM, Gheorghe A, Mvenge L, Gilbert C. Cost-effectiveness of eye care services in Zambia. *Cost Eff Resour Alloc* 2014; 12: 6.
- Agarwal A, Kumar DA. Cost-effectiveness of cataract surgery. *Curr Opin Ophthalmol* 2011; 22(1): 15–8.
- Eye Care Comparative Effectiveness Research Team (ECCERT). Hiratsuka Y, Yamada M, Akune Y, Murakami A, Okada AA, Yamashita H, et al. Cost-utility analysis of cataract surgery in Japan: a probabilistic Markov modeling study. *Jpn J Ophthalmol* 2013; 57(4): 391–401.
- Lansingh VC, Carter MJ, Martens M. Global cost-effectiveness of cataract surgery. *Ophthalmology* 2007; 114(9): 1670–8.
- Baltussen R, Sylla M, Mariotti SP. Cost-effectiveness analysis of cataract surgery: a global and regional analysis. *Bull World Health Organ* 2004; 82(5): 338–45.
- Nowak MS, Grabska-Liberek I, Michalska-Matecka K, Grzybowski A, Kozioł M, Niemczyk W, et al. Incidence and Characteristics of Cataract Surgery in Poland, during 2010-2015. *Int J Environ Res Public Health* 2018; 15(3): 435.
- Randleman JB, Wolfe JD, Woodward M, Lynn MJ, Chervenak DH, Srivastava SK. The Resident Surgeon Phacoemulsification Learning Curve. *Arch Ophthalmol* 2007; 125(9): 1215–9.

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## Discriminant validity of the Structured Inventory of Malingered Symptomatology (SIMS) under conditions of simulating symptoms

Diskriminativna validnost Strukturiranog inventara simuliranih simptoma (*Structured Inventory of Malingered Symptomatology* – SIMS) u uslovima simuliranja simptoma

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### Abstract

**Background/Aim.** The Structured Inventory of Malingered Symptomatology (SIMS) is a self-report measure to be used with adults, which may be utilized to assess the potential malingering of psychosis, neurologic impairment, amnesia, low intelligence, or affective disorder. The aim of the study was to examine the discriminant validity of SIMS under conditions of simulating symptoms of neurological and memory disorders, in response to a hypothetical situation, after watching a recording of an actual car accident in which a motorcycle rider sustained head injuries. **Methods.** The study involved 94 students (35 men and 59 women) from the University of Criminal Investigation and Police Studies in Belgrade and the Faculty of Medicine – Special Education and Rehabilitation in Novi Sad, aged 20–26 [arithmetic mean = 20.69; standard deviation (SD) = 0.80], divided into two groups (n = 47), malingerer and control. The malingerer group was instructed to identify with the motorcycle rider hit by the car and malingering symptoms related to neurological difficulties and amnesia in order to obtain greater reim-

bursement from the insurance company. The control group had instructions to honestly assess the probability of occurrence of the symptoms. **Results.** The results of the multivariate one-way analysis of variance suggested that the effect of experimental manipulation was statistically significant [ $F(88, 5) = 91.21, p < 0.001; \eta^2p = 0.838$ ]. Univariate effects were also statistically significant for all five scales. Participants in the malingerer group scored higher on all five scales than participants in the control group. The magnitudes of the effects support the largest differences between the malingerer and control groups on the scales of Memory Disorders and Neurological Disorders, which was also the basic instruction for simulating symptoms given to the participants in the malingerer group. **Conclusion.** The obtained results support the discriminant validity of the SIMS questionnaire in the situation of simulating symptoms of neurological disorders and memory disorders.

**Key words:** accidents, traffic; forecasting; insurance, liability; models, theoretical; surveys and questionnaires.

### Apstrakt

**Uvod/Cilj.** Strukturisani inventar simuliranih simptoma – *Structured Inventory of Malingered Symptomatology* (SIMS) je mera samoprocene koja se koristi kod odraslih osoba, a može biti korišćena za procenu potencijalnog razvoja psihoze, neurološkog oštećenja, amnezije, niske inteligencije ili afektivnog poremećaja. Cilj rada bio je da se ispita diskriminativna validnost SIMS-a u uslovima simuliranja simptoma neuroloških oštećenja i poremećaja pamćenja u odgovoru na hipotetičku situaciju, nakon gledanja snimka realne saobraćajne nezgode u kojoj je vozač motocikla zadobio povrede glave. **Metode.** U istraživanju su učestvovala 94 studenta (35 muškaraca i 59 žena) Kriminalističko-policijskog univerziteta u Beogradu i Medicinskog fakulteta – smer Specijalna edukacija i rehabilitacija u No-

vom Sadu, starosti od 20–26 godina [aritmetička sredina = 20.69; standardna devijacija (SD) = 0.80]), koji su bili podeljeni u dve grupe (n = 47), kontrolnu grupu i grupu ispitanika koji su simulirali simptome. Grupa ispitanika koji su simulirali simptome imala je zadatak da se poistoveti sa motociklistom kojeg je udario automobil i da simulira neurološke simptome i amneziju, sa ciljem da dobiju više novca od osiguravajuće kompanije. Kontrolna grupa imala je zadatak da iskreno proceni koji simptomi bi mogli nastati nakon saobraćajne nesreće. **Rezultati.** Rezultati multivarijantne jednosmerne analize varijanse su pokazali da je efekat eksperimentalne manipulacije bio statistički značajan [ $F(88, 5) = 91.21, p < 0.001; \eta^2p = 0.838$ ]. Univarijantni efekti su takođe bili statistički značajni za svih pet skala. Ispitanici iz kontrolne grupe postizali su niže skorove na svih pet skala u odnosu na ispitanike iz grupe koja je sim-

ulirala simptome. Veličine efekata govore u prilog najvećih razlika između grupe koja je simulirala simptome i kontrolne grupe na skalama Poremećaji pamćenja i Neurološka oštećenja, što je ujedno i bila osnovna instrukcija za simuliranje simptoma kod te grupe ispitanika. **Zaključak.** Dobijeni rezultati idu u prilog diskriminativne

validnosti upitnika SIMS u situaciji simuliranja simptoma neuroloških oštećenja i poremećaja pamćenja.

#### **Ključne reči:**

**udesi, saobraćajni; predviđanje; osiguranje, odgovornost; modeli, teorijski; ankete i upitnici.**

## **Introduction**

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), malingering is defined as “the intentional production of false or grossly exaggerated physical or psychological symptoms, motivated by external incentives”<sup>1</sup>. The symptoms of malingering are under voluntary control, with the emphasis placed on the awareness that the malingerer has in his/her presentation of malingering<sup>2-3</sup>. Therefore, malingering is based on understanding the symptoms of the disease, condition, and disorder rather than actual physical or psychological symptoms<sup>4-5</sup>. There is a significant difference between malingering psychopathology and neuropsychological deficiencies because, in the case of psychopathology, a person has to act out symptoms that are not present, and in organic deficits, one has to negate their ability and make deliberate mistakes<sup>6</sup>.

Although malingering is not a mental disorder, it has strong implications for both clinical and forensic practice. From the perspective of forensic practice, it is most common to malingering cognitive deficits, amnesia, as well as psychiatric, psychological, or physical symptoms<sup>7</sup>. In this context, malingering may aim to exclude liability for a crime<sup>8</sup>, through the exclusion of guilt, to obtain benefits through the payment of damages caused by a crime, or to avoid formal duty or responsibility<sup>1,9</sup>. In the first case, the malingerer malingeres all those symptoms that, in their opinion, should portray them as unaccountable, both at the time of the crime (schizophrenia, epilepsy, etc.) and after the crime (melancholy, mania, symptoms of concussion, etc.). In the latter case, malingerers simulate some physical disabilities that do not prevent them from performing certain actions. The forensic practice also encounters the simulation of amnesia, which usually occurs after the commission of violent criminal offenses, such as murder.

Regarding the basic rate of malingering, the results of the studies in Anglo-Saxon countries show that the frequency of malingering in forensic conditions is significant and ranges from 15.7%<sup>10</sup> to 45%<sup>11</sup>, where as many as 20%–30% of cases involve malingering of personal injury<sup>12-14</sup>. In criminal proceedings, malingering is present in about 19% of all cases<sup>15</sup>, out of which 20%–45% of cases involve defendants claiming amnesia related to the murder crime<sup>16-19</sup>.

For malingering assessment, several instruments have been developed, such as structured interviews, general psychological or cognitive instruments, and questionnaires specifically designed to identify malingering have also been constructed<sup>20</sup>.

The Structured Inventory of Malingered Symptomatology (SIMS) is a multidimensional questionnaire designed to

evaluate the symptoms of “false” psychopathology and cognitive function deficits and includes five scales<sup>21</sup>. The Psychosis (P) scale consists of 15 items that maintain the degree to which the respondent reports bizarre or unusual psychotic symptoms that are typically not present in actual psychiatric patients. The Neurological Impairment (NI) scale consists of 15 items that assess the degree to which the subject states illogical or very atypical neurological symptoms. The Memory Disorders (MD) scale consists of 15 items that indicate the degree to which the respondent lists symptoms of memory impairment that are not in line with the patterns of disorders found in injuries or brain dysfunction. The Low Intelligence (LI) scale consists of 15 items that assess the degree to which the respondent is trying to pretend to have general cognitive impairment or intellectual deficit. Finally, the Affective Disorders (AD) scale consists of 15 items that reflect the degree to which the respondent states atypical symptoms of depression or anxiety. The purpose of developing this inventory was to construct a psychometrically valid and cost-effective malingering assessment tool across domains applicable in clinical and forensic settings. The first stage of the development of this inventory involved the development of items that would be categorized into different categories of pathology, while the second stage involved psychometric improvement of the instrument<sup>22</sup>. The final version of the SIMS was empirically verified on a nonclinical sample of 476 students who joined the research voluntarily<sup>22</sup>. The results of this study indicated thresholds of  $P > 1$ ,  $NI > 2$ ,  $MD > 2$ ,  $LI > 2$ , and  $AD > 5$  as values that optimally differentiate non-malingering from malingering participants on each of the scales. Subsequently, these results were repeated using a cross-validation sample. It was concluded that the scores on the individual SIMS scales, as well as the overall score on the SIMS, had a high level of success (94.54%) in distinguishing the persons who engaged in malingering from those who responded genuinely. In other words, it has been found that respondents with a score higher than 14 were to be considered malingerers, and further assessment should be carried out given the large number of atypical, unlikely, inconsistent, or illogical symptoms reported by the malingerers<sup>21</sup>.

In the studies conducted mainly in the Netherlands, in which the respondents were mostly students<sup>22-27</sup>, the general conclusion was that the SIMS could provide valid data on the probable presence of malingering, indicated by thresholds greater than 14 or 16<sup>28</sup>.

In studies conducted mainly in the United States and some European countries, a design with well-known groups was applied. The samples consisted of respondents involved in legal proceedings, claimants, defendants, or inmates of a penal institution<sup>27, 29-33</sup>; the SIMS was found to be valid in

the process of discriminating between malingerers and those who answered the questions in the questionnaire honestly. The results of these studies suggest that the cut-off value ranged from  $> 14$  and  $> 16$ <sup>28</sup>, while in the studies of Clegg et al.<sup>34</sup>, it ranged from  $> 19$ , and in Wisdom et al.<sup>35</sup>, it was  $> 24$ .

The results of some research<sup>35–40</sup> conducted in real-world settings (clinical, forensic) support the constructive validity of the SIMS and the usefulness of its application in clinical and forensic settings. Yet it has been particularly emphasized that the SIMS should not be used as a stand-alone measure in clinical and forensic settings but rather in combination with other instruments covering different domains of symptomatology.

In the scientific work that follows, the main goal was to examine the discriminant validity of the SIMS questionnaire in the situation of malingering symptoms in experimental conditions. The importance of this research problem is reflected in the fact that there are no studies at the national level that test the validity of this questionnaire. The survey seeks to answer two questions (1) whether this inventory can identify respondents prone to malingering and (2) to what extent the SIMS questionnaire is sensitive to malingering symptoms. The answers to these questions are an important step in verifying the validity of this instrument, but may also indicate the usefulness of its application in national research as well as practical work (clinical or forensic assessment) because it is a relatively new measuring instrument that has only recently become available in Serbian.

## Methods

### *Sample and procedure*

The study involved 94 students (35 male and 59 female) from the University of Criminal Investigation and Police Studies in Belgrade and the Faculty of Medicine – Special Education and Rehabilitation in Novi Sad, Serbia, aged 20–26 [arithmetic mean = 20.69; standard deviation (SD) = 0.80]. The participants were divided into two groups ( $n = 47$ ), malingering and control, according to the criterion that every other participant was classified as a control group. The groups were uniform in relation to the faculty at which the participants study [ $\chi^2(1) = 2.31, p > 0.05$ ] as well as in terms of age [ $t(92) = 0.128, p > 0.05$ ] and gender [ $\chi^2(1) = 3.49, p > 0.05$ ]. The research was conducted in March 2019 in Belgrade and Novi Sad. The test conditions were identical for both groups, who individually completed the SIMS questionnaire after receiving the same instructions and after watching a recording of a real-life car accident in which a motorcycle rider sustained head injuries. The students of the University of Criminal Investigation and Police Studies first completed the questionnaire on the premises of the University of Criminal Investigation and Police Studies, while the students of the Faculty of Medicine completed the questionnaire on the premises of the Faculty of Medicine. The control group was instructed to independently evaluate the answer that was correct for them, that is, to honestly answer all the

items from the SIMS questionnaire. The malingering group was instructed to identify with the motorcycle rider and malingering symptoms related to neurological difficulties and amnesia in order to obtain greater reimbursement from the insurance company.

### *Research design*

The research design can be characterized as a one-factor multivariate experimental design without repetition. The independent variable has two levels: malingering and control group. The advantage of applying the experimental design in the context of this research was reflected in the possibility of applying different instructions to respondents from both groups. In other words, if experimental manipulation exerts a significant effect on dependent variables, the discriminant validity of the SIMS inventory is confirmed directly. The dependent variables in this study represented five scales of the SIMS questionnaire: NI, AD, P, LI, and MD.

### *Instrument*

SIMS<sup>21</sup> is a multidimensional questionnaire consisting of 75 items with a binary answer format (Yes/No) and items comprising five scales. NI scale includes 15 questions,  $\alpha = 0.945$ , and contains items related to illogical or atypical neurological impairment. AD scale includes 15 questions,  $\alpha = 0.846$ , and covers questions related to malingering atypical symptoms of anxiety or depression. P scale includes 15 questions,  $\alpha = 0.912$ , and measures the presence of bizarre or unusual symptoms that are not typically present in psychiatric patients. LI scale contains 14 questions,  $\alpha = 0.620$ , and includes items designed to assess the degree to which a respondent simulates general cognitive disability or cognitive deficit. Finally, the MD scale contains 15 questions,  $\alpha = 0.973$ , and includes items that relate to symptoms of certain memory problems and difficulties, that is, symptoms typical of head injuries. The translation and license for the application of this inventory were provided by Synapse Edition<sup>41</sup>. The translation of the inventory into Serbian was done using the standard back translation method. The translated version of the inventory was proofread and approved by two independent reviewers.

## Results

The results of descriptive statistics for the whole sample as well as for both groups are presented in Table 1. Arithmetic means and SD were consistently higher in the malingering group for all five scales of the SIMS questionnaire. The largest deviations from the normal distribution (conventionally acceptable values in the range of  $\pm 1.5$ <sup>42</sup>) were noticeable on the P scale at the whole sample level, as well as on LI and MD scales in the case of the control group. Concerning the distribution of scores within the malingering group, all scales were normally distributed, which was expected given the instruction given to the respondents before completing the questionnaire.

**Table 1**  
**Descriptive statistical parameters for the whole sample and both groups separately**

Scales	Whole sample				Control group				Malingerer group			
	M	SD	SK	KU	M	SD	SK	KU	M	SD	SK	KU
NI	5.39	4.99	0.62	-1.20	1.32	0.96	1.63	4.57	9.47	3.93	-0.48	-0.77
AD	5.20	3.12	0.62	-0.52	4.04	2.17	0.83	0.79	6.36	3.50	0.07	-1.14
P	2.36	3.57	2.01	3.23	0.96	0.91	0.81	0.08	3.77	4.58	1.04	-0.22
LI	5.64	1.88	-0.14	-0.21	4.60	1.65	0.02	-0.91	6.68	1.48	-0.05	1.54
MD	6.39	5.52	0.30	-1.71	1.47	1.06	2.49	7.13	11.32	3.30	-1.39	1.42

NI – neurological impairment; AD – affective disorders; P – psychosis; LI – low intelligence; MD – memory disorders; M – arithmetic mean; SD – standard deviation; SK – skewness; KU – kurtosis.

Correlations of all five scales of the SIMS inventory, for the whole sample as well as for both groups, are presented in Table 2. A consistent pattern of correlation was observed between AD and P scales, and this correlation is moderate, positive, and statistically significant. The correlation between the P and LI scales is significant across the sample as well as the groups, but the direction of correlation in the control group is negative, while in the remaining cases, it is positive, with a correlation moderate and significant statistically. Speaking generally, the correlations of the SIMS questionnaire scales are higher within the malingerer group.

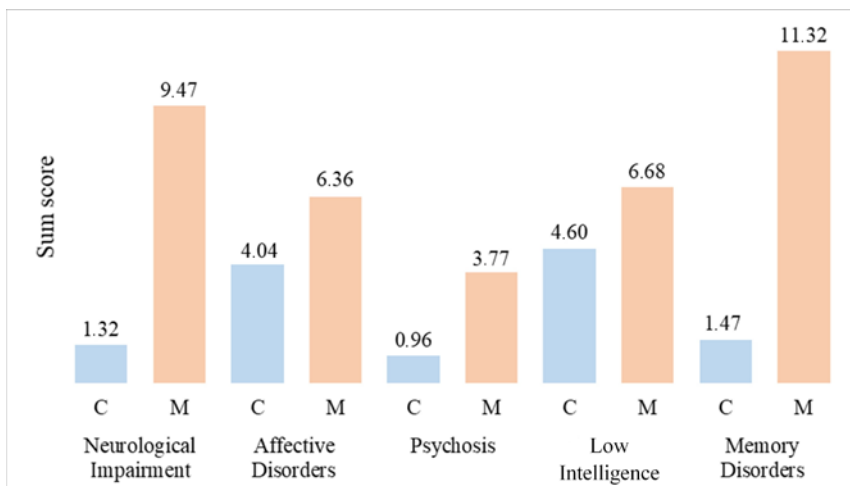
The results of the multivariate one-way analysis of variance suggest that the multivariate effect, i.e., the effect of experimental manipulation, was statistically significant [ $F(88, 5) = 91.21, p < 0.001; \eta^2p = 0.838$ ]. Univariate effects were also statistically significant for all five scales: [NI:  $F(93, 1) = 190.43, p < 0.001, \eta^2p = 0.674$ ; AD:  $F(93, 1) = 14.93, p < 0.001, \eta^2p = 0.140$ ; P:  $F(93, 1) = 17.01, p < 0.001, \eta^2p = 0.156$ ; LI:  $F(93, 1) = 41.68, p < 0.001, \eta^2p = 0.312$ ; MD:  $F(93, 1) = 380.01, p < 0.001, \eta^2p = 0.805$ ].

Participants in the malingerer group scored higher on all five scales than participants in the control group. Concerning the criteria for interpreting effect sizes proposed by Cohen<sup>43</sup>, all effects can be characterized as large. The largest differences between the malingerer and control groups were identified on the scales of MD and NI. The sum scores of both groups on all five scales of the SIMS questionnaire are presented in Figure 1. Due to deviations of individual scales from the normal distribution (Table 1), differences between groups on scales of the SIMS questionnaires were also tested using the Man-Whitney test (Table 3). The outcome of the application of parametric and nonparametric analysis is identical; the

**Table 2**  
**Relationship between Structured Inventory of Malingered Symptomatology (SIMS) questionnaire scales across the whole sample and both groups**

Scales	Whole sample				Control group				Malingerer group			
	NI	AD	P	LI	NI	AD	P	LI	NI	AD	P	LI
AD	0.253*				0.077				-0.136			
P	0.315**	0.580**			0.291*	0.366*			-0.032	0.563**		
LI	0.578**	0.275**	0.342**		-0.150	-0.074	-0.360*		0.431**	0.204	0.323*	
MD	0.846**	0.266**	0.319**	0.562**	0.128	0.379**	0.383**	-0.014	0.459**	-0.283	-0.119	0.267

NI – neurological impairment; AD – affective disorders; P – psychosis; LI – low intelligence; MD – memory disorders.  
\* < 0.05; \*\* < 0.01; \*\*\* < 0.001.



**Fig. 1 – Differences between control and malingerer groups on Structured Inventory of Malingered Symptomatology (SIMS) questionnaire scales. C – control group; M – malingerer group.**

**Table 3**  
**Differences between the control and malingering groups**  
**tested by the Mann-Whitney *U* test**

Scale	Group	MR	Z	<i>p</i>
Neurological impairment	C	25.40		
	M	69.60	-7.98	0.000
Affective disorders	C	38.66		
	M	56.34	-3.17	0.002
Psychosis	C	41.69		
	M	53.31	-2.14	0.032
Low intelligence	C	32.66		
	M	62.34	-5.36	0.000
Memory disorders	C	24.77		
	M	70.23	-8.33	0.000

**C – control group; M – malingering group; MR – mean ranks.**

participants in the malingering group achieved significantly higher scores than the control group on all five scales. In terms of percent of participants who simulated symptoms, results were very similar. In malingering group, 97.9% of participants simulated symptoms on the total SIMS score, 97.9% on the P scale, 95.7% on the NI scale, 97.9% on the MD scale, 100% on the LI scale, and 61.7% on the AD scale. In the control group, 23.4% of participants simulated symptoms on the total SIMS score, 21.3% on the P scale, 6.4% on the NI scale, 12.8% on the MD scale, 91.5% on the LI scale, and 25.5% on the AD scale.

### Discussion

The main objective of this study was to determine the discriminant validity of the SIMS questionnaire in the malingering of neurological symptoms and amnesia. Symptoms of those two scales best represent symptoms that may occur after the traffic/car accident. The results indicate that the most pronounced differences between the control and malingering participants were on the mentioned scales. This finding is expected since it stems directly from an experimental manipulation and speaks directly in favor of the discriminatory validity of the SIMS inventory, which is consistent with the results of foreign research<sup>22, 26, 35, 36, 38, 44</sup>. In other words, the SIMS adequately differentiates honest respondents from those who malingering neurological symptoms and memory deficits. The high sensitivity of this inventory is also supported by the magnitude of the effects of differences between the groups, which are very high for the two scales mentioned.

On the other hand, the malingering and control groups also differ on the remaining three SIMS scales – LI, AD, and P, with effects ranging from moderate to high. The results obtained were not expected because of the instruction given to the participants in the malingering group and can be explained in several ways. On the one hand, the results obtained can be explained by the ignorance of the symptoms included in the specific scales of the SIMS inventory on the part of the students who participated in this research, especially when it comes to students of the University of Criminal Investigation and Police Studies. In other words, the formal education of students does not include comprehensive training in clinical psychology, which made it impossible for

the respondents in the malingering group to precisely identify which symptoms relate to scales of MD and ND and which do not relate to the scales mentioned. Another potential explanation is the tendency towards over-generalization and reporting of different symptoms, as indicated by the results of previous studies<sup>43–46</sup>. The third alternative explanation for the results obtained relates to the subjective beliefs of the respondents regarding which symptoms can be malingered after a road traffic accident, with the conclusion that these symptoms do not necessarily relate to the scales mentioned. Furthermore, the differences obtained on the remaining three SIMS scales (AD, P, LI) can be understood as a very high discriminant validity/sensitivity of the SIMS questionnaire, which is also an advantage of this inventory, as it can identify subtler, i.e., less pronounced forms of malingering.

### *Limitations and guidelines for future research*

The scenario used in the research is considered an experimental malingering model. Thus, in experimental conditions, certain symptoms of the malingering participants responded to a hypothetical rather than a realistic situation<sup>22</sup>. Against this background, a guideline for future research is to test the validity of the SIMS inventory in the general population under realistic malingering conditions to examine the ecological validity of this inventory. The importance of the abovementioned proposal for further research is reflected in the fact that, despite the existing studies that have applied the design with known groups, there is still a need for a more precise determination of the ecological validity of the SIMS questionnaire.

Although most simulation studies assume that the respondents will have appropriate motivation<sup>42</sup>, such an assumption needs to be verified in prospective studies. In other words, the assumption that respondents behave credibly<sup>43</sup> in a malingering situation as well as in a real situation should be verified by empirical methods.

As the malingering and control groups differ on all scales of the SIMS questionnaire, the recommendation for future research is to provide malingeringers with sufficient time and information to familiarize themselves with the symptoms of specific scales and to test the malingeringers' knowledge of the symptoms they have to malingering. In this way, alternative in-

interpretations of the results obtained can be avoided. Additional limitations of this research are the small sample size and lack of information on whether the respondents or their relatives had experience with traffic accidents.

#### *Recommendations to practitioners*

In line with the results of previous studies, it was found that the SIMS questionnaire has satisfactory validity in the situation of malingering the symptoms of neurological damage and memory impairment. Given the above, as well as the fact that its administration and interpretation<sup>28</sup> are easy, the SIMS questionnaire could be used in practice as a very convenient screening instrument<sup>22</sup>. Although the assumption of sincerity may be unfounded, especially in the forensic context, the burden of proof regarding the existence of malingering is still on the experts who will use this instrument<sup>28, 47</sup>. The high overall score on the SIMS, as well as the high limit values on the individual SIMS scales, do not satisfy the burden of proof but should be an incentive for further evaluation regarding the presence of malingering<sup>28</sup>.

When using the SIMS inventory in practice, it is important to emphasize the possible occurrence of two types of errors: false-positive errors, in which a person is classified as a simulant, while being a real patient, and false-negative errors, in which a person is classified as a *bona fide* patient, while being a simulant<sup>6</sup>. In the case of a false positive, it could result in a violation of civil rights, that is, a conviction and imposition of an unjustified prison sentence if the individual is found guilty. There are also other implications, such as not getting the necessary psychiatric help, disability benefits, etc. In the case of a false negative, a person may receive unnecessary psychiatric or medical assistance or unjustified financial compensation or compensation for damage. Finally, as with any clinical method or procedure, the usefulness and validity of the SIMS depend on the qualification and competence of the professionals using this instrument.

The use of the SIMS in combination with other instruments such as the Symptom Validity Tests (SVTs) or Per-

formance Validity Tests (PVTs)<sup>28</sup> in the context of a comprehensive evaluation is consistent with Hutchinson's<sup>48</sup> recommendation that malingering disorders should be determined multiple times and that they require a multidimensional discovery strategy. At the same time, using the SIMS in combination with other tests designed to detect malingering allows a significant reduction in false-positive errors since the subject must "fail" at least two tests in order to qualify as a simulant<sup>45</sup>. Thus, it is essential that symptom validity assessment involves multiple measures covering different domains of symptomatology during different stages of evaluation<sup>48, 49</sup>.

We also believe that the SIMS questionnaire should be supplemented by conducting a structured interview, even though this method is time-consuming and requires a trained assessor. This is also the recommendation of some researchers<sup>49-52</sup> who have dealt with the problem of disorder assessment and malingering symptoms. That would reduce or eliminate possible evaluation errors previously noted in making diagnostic decisions<sup>51</sup>. When conducting an interview, the examiner should pay particular attention to the exaggeration and dramatic presentation of symptoms<sup>9</sup>, inconsistencies regarding psychiatric diagnosis, and reporting of rare, atypical, or extreme symptoms<sup>6</sup>.

#### **Conclusion**

The main contribution of this study could be divided into two important aspects. First, this is the very first study that aimed to validate the SIMS inventory in our country prior to our knowledge. Second, our results show that the SIMS inventory can detect the simulation of different symptoms in a hypothetical situation. Altogether, results from previous studies and this study indicate that the SIMS inventory can be used for detecting the simulation of different symptoms in both real and hypothetical situations. In addition, our study has shown that this instrument can be used in practice as a reliable measure of the simulation of symptoms in our country.

#### R E F E R E N C E S

1. *American Psychiatric Association*. Diagnostic and Statistical Manual of Mental Disorders. (DSM-5). 5th ed. Washington, DC: American Psychiatric Association Publishing; 2013.
2. *Merkelbach H, Boskovic I, Pesy D, Dalskelev M, Lynn SJ*. Symptom overreporting and dissociative experiences: A qualitative review. *Conscious Cogn* 2017; 49: 132-44.
3. *Sadock BJ, Sadock VA, Ruiz P*, editors. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia, PA: Lippincott Williams and Wilkins/Wolter Kluwer; 2009.
4. *Keyvan A, Ger MC, Ertürk SG, Türkan A*. The validity and reliability of the Turkish version of the Miller Forensic Assessment of Symptoms Test (M-FAST). *Noro Psikiyatrs Ars* 2015; 52(3): 296-302.
5. *Kožarić-Kovačić D, Borovečki A, Udovičić S, Kocijan-Hervegona*. Malingered PTSD. *Drušvena istraživanja* 2003; 12(3-4): 541-59. (Croatian)
6. *Rogers R*. Development of a new classificatory model of malingering. *Bull Am Acad Psychiatry Law* 1990; 18(3): 323-33.
7. *Gudjonsson HG*. Interrogative suggestibility: Its relationship with assertiveness, social-evaluative anxiety, state anxiety and method of coping. *Brit J Clin Psychol* 1988; 27(2): 159-66.
8. *Resnick PJ*. Guidelines for evaluation of malingering in PTSD. In: *Simon RI*, editors. *Posttraumatic stress disorder in litigation*. 2nd ed. Washington, DC: American Psychiatric Publishing; 2003. p. 187-205.
9. *Kardum-Skelin I, Turek PJ*. Testis and scrotum: cytology of testicular and scrotal masses and male infertility. In: *Gray W, Kojan G*, editors. *Diagnostic Cytopathology*. 3rd ed. London: Churchill Livingstone, Elsevier; 2010. p. 585-600.
10. *Boskovic I, van der Heide D, Hope L, Merkelbach H, Jelicic M*. Plausibility Judgments of Atypical Symptoms Across Cultures: an Explorative Study Among Western and Non-Western Experts. *Psychol Inj Law* 2017; 10(3): 274-81.

11. Rogers R, Sewell KW, Goldstein AM. Explanatory models of malingering: A prototypical analysis. *Law Hum Behav* 1994; 18(5): 543–52.
12. Noriss MP, May MC. Screening for malingering in a correction setting. *Law Hum Behav* 1998; 22(3): 315–23.
13. Lees-Haley PR. MMPI-2 base rates for 492 personal injury plaintiffs: Implications and challengers for forensic assessment. *J Clin Psychol* 1997; 53(7): 754–55.
14. Green P, Robling ML, Lees-Haley PR, Allen LM. Effort has a greater effect on test scores than severe brain injury in compensation claimants. *Brain Injury* 2001; 15(12): 1045–60.
15. Langeluddecke PM, Lucas SK. Quantitative measures of memory malingering on the Wechsler Memory Scale-third edition in mild head injury litigants. *Arch Clin Neuropsychol* 2003;18(2): 181–97.
16. Mittendberg W, Patton C, Canyock EM, Condit DC. Base rates of malingering and symptom exaggeration. *J Clin Exp Neuropsychol* 2002; 24(8): 1094–102.
17. Kopelman MD. Amnesia: Organic and psychogenic. *Br J Psychiatry* 1987; 150: 428–42.
18. Kopelman MD. The assessment of psychogenic amnesia. In: *Baddeley AD, Wilson BA, Watts FN*, editors. *Handbook of memory disorders*. Chichester: Wiley; 1995. p. 427–48.
19. Schacter DL. Amnesia and crime: How much do we really know? *Am Psychol* 1986; 41(3): 286–95.
20. Taylor PJ, Kopelman MD. Amnesia for criminal offences. *Psychol Med* 1984; 14(3): 581–8.
21. Smith GP. Assessment of malingering with self-report instruments. In: *Rogers R*, editor. *Clinical assessment of malingering and deception*. 2nd ed. New York: Guilford Press; 1997. p. 351–70.
22. Smith GP, Burger GK. Detection of malingering: Validation of the Structured Inventory of Malingered Symptomatology (SIMS). *J Am Acad Psychiatry Law* 1997; 25(2): 183–9.
23. Dandachi-FitzGerald B, Merckelbach H. Feigning ≠ Feigning a Memory Deficit: The Medical Symptom Validity Test as an Example. *J Exp Psychopathol* 2013; 4(1): 46–63.
24. Jelicic M, Ceunen E, Peters MJV, Merckelbach H. Detecting coached feigning using the Test of Memory Malingering (TOMM) and the Structured Inventory of Malingered Symptomatology (SIMS). *J Clin Psychol* 2011; 67(3): 850–5.
25. Jelicic M, van Gaal M, Peters MJV. Expert knowledge doesn't help: Detecting feigned psychosis in people with psychiatric expertise using the Structured Inventory of Malingered Symptomatology (SIMS). *J Exp Psychopathol* 2013; 4(1): 38–45.
26. Merckelbach H, Collaris J. Mother Theresa doesn't help here: Lack of moral priming effects on malingered symptom reports and what we can learn from it. *Psychol Belg* 2012; 52(3): 271–85.
27. Rogers R, Robinson EV, Gillard ND. The SIMS screen for feigned mental disorders: The development of detection-based scales. *Behav Sci Law* 2014; 32(4): 455–66.
28. Vossler-Thies E, Stevens A, Engel RR, Licha C. Erfassung negativer Antwortverzerrungen mit der deutschen Fassung des "Personality Assessment Inventory", dem "Verhaltens-und Erlebensinventar". *Diagnostica* 2013; 59(2): 73–85.
29. van Impelen A, Merckelbach H, Jelicic M, Merten T. The Structured Inventory of Malingered Symptomatology (SIMS): A Systematic Review and Meta-Analysis. *Clin Neuropsychol* 2014; 28(8): 1336–65.
30. Alves YR, Clark JA, Berry DTR, Granacher RP. Screening for feigning in a civil forensic setting. *J Clin Exp Neuropsychol* 2008; 30(2): 133–40.
31. González Ordi H, Santamaría Fernández P. Detection of malingering in clinical, medicolegal, and forensic settings. In: *González Ordi H, Santamaría Fernández P*, editors. *Inventario Estructurado de Simulación de Síntomas – The SIMS Manual*. Madrid: Tea Ediciones; 2008. p. 60–6.
32. Green D, Rosenfeld B. Evaluating the gold standard: A review and meta-analysis of the Structured Interview of Reported Symptoms. *Psychol Assess* 2011; 23(1): 95–107.
33. Lewis JL, Simcox AM, Berry DTR. Screening for feigned psychiatric symptoms in a forensic sample by using the MMPI-2 and the Structured Inventory of Malingered Symptomatology. *Psychol Assess* 2002; 14(2): 170–6.
34. Clegg C, Fremouw W, Mogge N. Utility of the Structured Inventory of Malingered Symptomatology (SIMS) and the Assessment of Depression Inventory (ADI) in screening for malingering among outpatients seeking to claim disability. *J Forensic Psychiatr Psychol* 2009; 20: 239–54.
35. Wisdom NM, Callahan JL, Shaw TG. Diagnostic utility of the Structured Inventory of Malingered Symptomatology to detect malingering in a forensic sample. *Arch Clin Neuropsychol* 2010; 25(2): 118–25.
36. Can Ardic F, Kose S, Solmaz M, Kalacaoglu F, Balcioglu YH. Reliability, validity, and factorial structure of the Turkish version of the Structured Inventory of Malingered Symptomatology (Turkish SIMS). *Psychiatry Clin Psychopharmacol* 2019; 29(2): 182–8.
37. De Marchi B, Balboni G. Detecting malingering mental illness in forensics: Known-Group Comparison and Simulation Design with MMPI-2, the SIMS and NIM. *PeerJ* 2018; 6: e5259.
38. Malcore SA, Schutte C, Van Dyke SA, Axelrod BN. The development of a reduced-item Structured Inventory of Malingered Symptomatology (SIMS). *Psychol Inj Law* 2015; 8(2): 95–9.
39. Roma P, Giromini L, Burla F, Ferracuti S, Viglione DJ, Mazzà C. Ecological Validity of the Inventory of Problems-29 (IOP-29): An Italian Study of Court-Ordered, Psychological Injury Evaluations Using the Structured Inventory of Malingered Symptomatology (SIMS) as Criterion Variable. *Psychol Inj Law* 2020; 13(1): 57–65.
40. Tabachnick BG, Fidell LS. *Using Multivariate Statistics*. 6th ed. MA Boston: Pearson; 2013.
41. Synapse Edition. Available from: <https://www.sinapsaedic.ije.rs/> (Serbian)
42. Rogers R, Cruise KR. Assessment of malingering with simulation designs: threats to external validity. *Law Hum Behav* 1998; 22(3): 273–85.
43. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. New York, NY: Routledge Academic; 1988.
44. Malcore SA, Schutte C, Van Dyke SA, Axelrod BN. The development of a reduced-item structured inventory of malingered symptomatology (SIMS). *Psychol Inj Law* 2015; 8(2): 95–9.
45. Giger P, Merten T, Merckelbach H, Oswald M. Detection of feigned crimelated amnesia: A multi-method approach. *J Forensic Psychol Pract* 2010; 10: 440–63.
46. Merten T, Lorenz R, Schlatow S. Posttraumatic Stress Disorder can easily be faked, but faking can be detected in most cases. *Ger J Psychiatry* 2010; 13(3): 140–9.
47. Rogers R. An introduction to response styles. In: *Rogers R*, editor. *Clinical Assessment of Malingering and Deception*. 3rd ed. New York: Guilford Press; 2008. p. 3–13.
48. Hutchinson GL. *Disorders of simulation: Malingering, factitious disorders, and compensation neurosis*. CT: Madison: Psychological Press; 2001.
49. Boone KB. The need for continuous and comprehensive sampling of effort/response bias during neuropsychological examinations. *Clin Neuropsychol* 2009; 23(4): 729–41.
50. Heilbronner RL, Sweet JJ, Morgan JE, Larrabee GJ, Millis SR. *Conference Participants. American Academy of Clinical Neuropsychology Consensus Conference Statement on*

the neuropsychological assessment of effort, response bias, and malingering. *Clin Neuropsychol* 2009; 23(7): 1093–129.

51. *DeClue G*. Practitioner's Corner: Feigning ≠ Malingering: A case study. *Behav. Sci. Law* 2002; 20: 717–26.
52. *Rogers R, Jackson RL, Salekin KL, Neumann CS*. Assessing Axis I symptomatology on the SADS-C in two correctional samples:

The validation of subscales and a screen for malingered presentations. *J Pers Assess* 2003; 81(3): 281–90.

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## Clinical measurement of maximum mouth opening and its relation to different facial types in children of Saudi Arabia

Kliničko merenje maksimalnog otvaranja usta i njegova povezanost sa različitim tipovima lica kod dece iz Saudijske Arabije

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### Abstract

**Background/Aim.** The decreased mouth opening (MO) is a key factor in diagnosing most oral health-related issues. The aim of the study was to assess the magnitude of the maximum mouth opening (MMO) and its correlation with the facial types of children in the southern part of Saudi Arabia. **Methods.** A cross-sectional study was conducted in a group of 555 children ranging from 2 to 12 years of age. The MMO was measured as the distance between the incisal edge of maxillary and mandibular central incisors in the midline using the digital Vernier caliper. Three readings of MMO value were obtained, and the average mean of these readings was taken as the final MMO value. Based on the calculated facial index (FI), five facial types were determined in the study subjects: hypereuryprosopic (very broad face, FI range  $\leq 79.9$ ); euryprosopic (broad face, FI range 80–84.9); mesoprosopic (round face, FI range 85–89.9); leptoprosopic (long face, FI range 90–94.9); hyperleptoprosopic (very long face, FI range  $\geq 95$ ). Subjects were categorized and compared accordingly. The facial profile, height,

weight, and body mass index (BMI) of each subject were recorded as well. The data hence obtained was subjected to statistical analysis using SPSS (version 19.0, SPSS Incorporated, Chicago, IL, USA). **Results.** The overall mean value  $\pm$  standard deviation of MMO for all the subjects in the study was  $47.3 \pm 8.7$  mm. The mean value of MMO in hyperleptoprosopic children (FI range  $\geq 95.0$ ) was significantly higher than in other children. The mean difference value of MO in mesoprosopic (FI range 85.0–89.9) children was significantly lower than in leptoprosopic (FI range 90.0–94.9) and hyperleptoprosopic (FI range  $\geq 95.0$ ) children. The mean difference value of MO of the leptoprosopic (FI range 90.0–94.9) type was significantly lower than that of the hyperleptoprosopic type (FI range  $\geq 95.0$ ). **Conclusion.** Based on the analyzed results, it was found that the MMO correlates with different facial types and facial profiles among the studied population.

**Key words:**  
anatomy; correlation of data; dental care for children; face; orthodontics; saudi arabia.

### Apstrakt

**Uvod/Cilj.** Smanjeno otvaranje usta (OU) je ključni faktor u dijagnostici većine problema povezanih sa oralnim zdravljem. Cilj studije bio je da se proceni veličina maksimalnog otvaranja usta (MOU) i njena korelacija sa tipovima lica kod dece u južnom delu Saudijske Arabije. **Metode.** Studija preseka obuhvatila je grupu od 555 dece od 2 do 12 godina. MOU mereno je kao rastojanje između incizalne ivice maksilarnih i mandibularnih centralnih sekutića na srednjoj liniji, korišćenjem digitalnog Vernierovog nonijusa. Prosečna srednja vrednost tri merenja MOU uzeta je kao konačna vrednost MOU. Na osnovu izračunatog indeksa lica (IL) utvrđeno je postojanje pet

tipova lica kod ispitanika: hipereuriprozop (veoma široko lice, IL raspon  $\leq 79,9$ ); euriprozop (široko lice, IL raspon 80–84,9); mezoprozop (okruglo lice, IL raspon 85–89,9); leptoprozop (dugačko lice, IL raspon 90–94,9); hiperleptoprozop (veoma dugačko lice, IL raspon  $\geq 95$ ). U skladu sa tim, ispitanici su kategorisani i izvršeno je poređenje. Takođe su procenjeni i tipovi profila lica, visina, težina i indeks telesne mase (ITM) ispitanika. Tako dobijeni podaci su podvrgnuti statističkoj analizi korišćenjem SPSS-a (verzija 19,0 SPSS Incorporated, Čikago, IL, SAD). **Rezultati.** Srednja vrednost  $\pm$  standardna devijacija za MOU izračunata na nivou celokupne grupe u studiji bila je  $47,3 \pm 8,7$  mm. Srednja vrednost MOU kod tipa hiperleptoprozopa (IL raspon  $\geq 95,0$ ) bila

značajno viša nego kod druge dece. Srednja razlika vrednosti OU kod tipa mezoprozopa (IL raspon 85,0–89,9) bila je značajno niža nego kod tipa leptoprozopa (IL raspon 90,0–94,9) i tipa hiperleptoprozopa (IL raspon  $\geq 95,0$ ) dece. Srednja razlika vrednosti OU kod tipa leptoprozopa (IL raspon 90,0–94,9) bila je značajno niža nego kod tipa hiperleptoprozopa (IL raspon  $\geq 95,0$ ) dece. **Zaključak.**

Analizom rezultata utvrđeno je da postoji povezanost MOU sa različitim tipovima i profilima lica u okviru proučavane populacije.

**Ključne reči:**  
anatomija; podaci, korelacija; deca, stomatološka nega; lice; ortodoncija; saudijska arabija.

## Introduction

The distance between the mesioincisal edge of the maxillary central incisors when the mouth is wide open, known as maximum mouth opening (MMO), also referred to as maximal interincisal distance<sup>1, 2</sup>, is an excellent parameter available in the assessment of the range of vertical mandibular movements<sup>3</sup>. The temporomandibular joint (TMJ) dysfunction and reduced mouth opening (MO) can be caused due to various conditions such as oral health-related infections, malignancies involving the craniofacial region, fractures of jaws, and myopathies in the head and neck region<sup>4</sup>. Moreover, MMO provides the requisite information for designing prostheses and dental appliances<sup>5</sup>.

There is no pertinent reference, and the clinicians are thus confronted with a somewhat difficult situation while assessing whether the MO is limited or normal<sup>6, 7</sup>; hence the correlation of MMO with other related physiological parameters holds prominence therein<sup>8</sup>. Previous researchers have observed the association of facial width with facial plastic surgeons, orthodontists, and maxillofacial surgeons<sup>9, 10</sup>. Researchers have also reported that MO varies with demographic characteristics such as age, race, gender, and body size (weight and height)<sup>11</sup>. Decreased MO is a key factor in diagnosing most oral health-related conditions; its early and timely detection is of great significance insofar as the treatment and management are concerned.

Given the facts above, a study was planned and carried out to assess the MMO and its correlation with facial types among children in southern Saudi Arabia.

## Methods

A cross-sectional study was conducted among the patients who reported to the Dental Clinics of King Khalid University College of Dentistry, Abha, Saudi Arabia. A convenient sample of 555 children was taken for the study, with the participants ranging from 2 to 12 years old.

Prior to conducting the study, ethical clearance was given from the Scientific Research Committee, King Khalid University College of Dentistry (IRB/KKUCOD/ETH/2020-21/013). Only those children whose parents/guardians gave consent for participation and who had no orofacial anomalies or history of trauma related to facial or dental structures were included in the study. Children with missing or fractured maxillary or mandibular incisors or suffering from any cranio-mandibular disorders, bruxism, or those who underwent any orthodontic treatment or had any history of temporomandibular disorder (TMD) were excluded from the study.

The subjects were told to sit in a straight upright position, head resting with back support, and were asked to open their mouths as wide as possible to measure the MMO. The MMO was measured as the distance between the incisal edge of maxillary and mandibular central incisors in the midline using the digital Vernier caliper (Digimatic caliper, Mitutoyo, UK). Two caliper instruments were calibrated for performing the clinical examination, and the inter-instrument and intra-instrument reliability was assessed using *kappa* statistics. The *kappa* coefficient for inter- and intra-instrument reliability was found to be 0.87 and 0.91, respectively. Three readings of MMO were obtained by each caliper, and the average mean of these readings was taken as the final MMO. To assess the facial type, the facial index (FI) was calculated using a formula: FI = morphological facial length between *nasion* (N) and *gnathion* (G), divided by morphological facial width between the left and right *zygion* (Z), multiplied by 100. N is the midpoint on the soft tissue contour of the base of the nasal root at the level of the frontonasal suture. G is the most inferior midline point on the soft tissue chin contour. Z is the most lateral point on the soft tissue contour of each zygomatic arch.

Morphological facial height was measured as a straight distance from N to G (the lowest mid-point of the mandible). Morphological facial width was measured as the widest distance between the zygomatic prominences. The most lateral point of the zygomatic arch was palpated on both sides, and the ends of the caliper were placed on these points for measurement. From these two values (morphological facial height and morphological facial width), the FI was determined.

Based on the calculated FI, five facial types were determined, and subjects were categorized and compared accordingly. Five facial types were as follows: hypereuryprosopic (very broad face, FI range  $\leq 79.9$ ); euryprosopic (broad face, FI range 80–84.9); mesoprosopic (round face, FI range 85–89.9); leptoprosopic (long face, FI range 90–94.9); hyperleptoprosopic (very long face, FI range  $\geq 95$ )<sup>12</sup>.

The facial profiles of the subjects were assessed and recorded. The facial profile was assessed by viewing the superficial facial features from the subject's left side while the subject was made to sit in a comfortable upright position. The facial profile was assessed by joining the following two reference lines: a line joining the forehead and the soft tissue point 'A' (deepest point in the curvature of the upper lip), and a line joining point 'A' and the soft tissue pogonion (most anterior point of the chin)<sup>13</sup>. Based on the relationship between these two lines, three facial profile types were recorded: a straight profile when the two lines form a nearly straight line, a convex profile when the two lines form an an-

gle with the concavity facing the tissue, and a concave profile when the two lines form an angle with convexity toward the tissue.

The height, weight, and body mass index (BMI) of the subjects were also recorded. The data hence obtained was subjected to statistical analysis using SPSS (version 19.0, SPSS Incorporated, Chicago, IL, USA).

## Results

Out of the total of 555 subjects, 184 were male children and 371 were female children ranging from 2 to 12 years of age with a mean age of  $4.99 \pm 3.15$  years. The overall mean value [ $\pm$  standard deviation (SD)] of MMO among the subjects was  $47.3 \pm 8.7$  mm. The mean value of MMO was  $49.5 \pm 0.5$  mm in males, while it was  $46.3 \pm 0.5$  mm in females, with a statistically significant difference between the two (Table 1). The MMO was bigger in the females than males at

all ages, with the exception of the ages of 2 and 3 years old. That was, however, statistically significant at 2, 3, 6, 7, 8, and 9 years of age ( $p < 0.01$ ) (Table 2). MMO was found to be 47.5 mm among those subjects with no TMJ abnormality, and it was 46.7 mm and 46.9 mm among those having clicking and deviation, respectively, with a statistically non-significant difference (Table 3).

It was found that the mean value of MMO of the hyperleptoprosopic type was significantly higher than in other types. The mean difference of MO of mesoprosopic type was significantly lower than leptoprosopic and hyperleptoprosopic type. The mean difference of MO of the leptoprosopic type was significantly lower than in the hyperleptoprosopic type (Table 4).

The MMO value was 50.2 mm for those having a concave facial profile, while it was 44.6 mm and 48.3 mm for those with convex and straight profiles, respectively, with statistically significant differences (Table 5).

**Table 1**

### Mean maximum mouth opening (MMO) according to gender

Gender	n	MMO (mm) mean $\pm$ SD	MMO difference (mm) mean $\pm$ SD	p-value
males	175	$49.5 \pm 0.5$		
females	380	$46.3 \pm 0.5$	$3.2 \pm 0.8$	0.000

SD – standard deviation.

**Table 2**

### Mean maximum mouth opening (MMO) according to age and gender

Age (years)	Gender	n	MMO (mm) mean (SD)	p-value
2	male	17	50.1 (1.6)	0.000**
	female	177	41.4 (0.5)	
3	male	21	52.6 (2.15)	0.003**
	female	105	45.9 (0.9)	
6	male	5	47.1 (1.4)	0.002**
	female	4	56.1 (1.2)	
7	male	30	47.9 (0.9)	0.000**
	female	4	59.7 (1.5)	
8	male	41	48.9 (0.7)	0.000**
	female	42	54.7 (1.4)	
9	male	57	49.3 (0.9)	0.004**
	female	20	54.6 (1.5)	
10	male	4	51.5 (2.9)	0.961
	female	9	51.3 (1.8)	
11	male	4	56.7 (1.5)	0.073
	female	6	60.7 (1.2)	
12	male	5	58.2 (3.8)	0.930
	female	4	58.7 (4.1)	

SD – standard deviation; \*\* – statistically highly significant.

**Table 3**

### Relation of maximum mouth opening (MMO) to transmandibular joint (TMJ) functioning

TMJ	n	MMO (mm) mean (SD)	95% CI for mean		Minimum (mm)	Maximum (mm)	p-value
			lower bound (mm)	upper bound (mm)			
NAD	340	47.5 (0.5)	46.5	48.4	25.3	70.3	
Clicking	128	46.7 (0.8)	45.1	48.2	34.0	67.0	0.746
Deviation	45	46.9 (1.1)	44.7	49.2	31.3	61.3	

CI – confidence interval; NAD – no abnormality detected; SD – standard deviation.

The correlation coefficient  $r$  of MMO with age (0.446) shows a significant moderate positive correlation ( $p < 0.01$ ), while the correlation coefficient  $r$  of MMO with weight (-0.090) shows a significant weak negative correlation ( $p < 0.05$ ). The correlation coefficient  $r$  of MMO with BMI (-0.096) shows a

significant weak negative correlation,  $p < 0.05$ . The correlation coefficient  $r$  of MMO with facial width (FW) (-0.087) shows a significant weak negative correlation,  $p < 0.05$ , while the correlation coefficient  $r$  of MMO with the facial type (0.107) shows a significant weak positive correlation,  $p < 0.01$  (Table 6).

Table 4

## Relation of maximum mouth opening (MMO) to facial types

Facial types (FI range)	n	MMO (mm) mean (SD)	95% CI for mean		Minimum (mm)	Maximum (mm)	p-value $\diamond$
			lower bound (mm)	upper bound (mm)			
Hypereuryprosopic ( $\leq 79.9$ )	11	48.0 (3.2)	40.9	55.1	34.0	70.3	
Euryprosopic (80.0–84.9)	49	43.3 (1.3)	40.6	46.1	25.3	65.3	
Mesoprosopic (85.0–89.9)	95	46.6 <sup>a</sup> (0.9)	44.9	48.4	32.0	67.0	0.010
Leptoprosopic (90.0–94.9)	111	47.7 <sup>b</sup> (0.9)	45.9	49.5	32.3	67.7	
Hyperleptoprosopic ( $\geq 95.0$ )	289	48.1 <sup>c</sup> (0.5)	47.1	49.0	25.7	71.2	

CI – confidence interval; FI – facial index; SD – standard deviation; a vs. b, a vs. c, and b vs. c is statistically significant;  $\diamond - p < 0.05$  by one-way ANOVA.

Table 5

## Relation of maximum mouth opening (MMO) to facial profiles

Facial profile	n	MMO (mm) mean (SD)	95% CI for mean		Minimum (mm)	Maximum (mm)	p-value
			lower bound (mm)	upper bound (mm)			
Convex	201	44.6 <sup>a</sup> (0.6)	43.4	45.8	30.7	67.7	0.000
Straight	266	48.3 <sup>b</sup> (0.5)	47.3	49.4	25.3	67.0	
Concave	88	50.2 <sup>c</sup> (0.7)	48.7	51.7	36.7	70.3	

CI – confidence interval; SD – standard deviation; a vs. b, a vs. c, and b vs. c is statistically significant.

Table 6

## Correlation of maximum mouth opening (MMO) (n = 555) with different parameters

		MMO Average	Age	Weight	Height	BMI	FL	FW	TMJ	Facial type
MMO Average	$r$	1	0.465**	-0.090*	-0.029	-0.096*	0.059	-0.087*	0.025	0.107*
	p-value		0.000	0.035	0.490	0.023	0.167	0.041	0.555	0.012
Age	$r$	0.465**	1	-0.228**	-0.178**	-0.107*	0.107*	-0.350**	0.034	0.346**
	p-value	0.000	/	0.000	0.000	0.011	0.012	0.000	0.425	0.000
Weight	$r$	-0.090*	-0.228**	1	0.707**	0.546**	0.348**	0.478**	0.069	-0.085*
	p-value	0.035	0.000	/	0.000	0.000	0.000	0.000	0.103	0.046
Height	$r$	-0.029	-0.178**	0.707**	1	-0.195**	0.430**	0.362**	0.033	0.060
	p-value	0.490	0.000	0.000	/	0.000	0.000	0.000	0.438	0.158
BMI	$r$	-0.096*	-0.107*	0.546**	-0.195**	1	-0.014	0.242**	0.048	-0.186**
	p-value	0.023	0.011	0.000	0.000	/	0.737	0.000	0.259	0.000
FL	$r$	0.059	0.107*	0.348**	0.430**	-0.014	1	0.117**	0.041	0.680**
	p-value	0.167	0.012	0.000	0.000	0.737	/	0.006	0.339	0.000
FW	$r$	-0.087*	-0.350**	0.478**	0.362**	0.242**	0.117**	1	0.024	-0.645**
	p-value	0.041	0.000	0.000	0.000	0.000	0.006	/	0.572	0.000
TMJ	$r$	0.025	0.034	0.069	0.033	0.048	0.041	0.024	1	0.011
	p-value	0.555	0.425	0.103	0.438	0.259	0.339	0.572	/	0.793
Facial type	$r$	0.107*	0.346**	-0.085*	0.060	-0.186**	0.680**	-0.645**	0.011	1
	p-value	0.012	0.000	0.046	0.158	0.000	0.000	0.000	0.793	/

BMI – body mass index; FL – facial length; FW – facial width; TMJ – transmandibular joint; \*\* correlation is significant at the  $p = 0.01$  level (2-tailed); \* correlation is significant at the  $p = 0.05$  level (2-tailed);  $r$  – Pearson correlation.

## Discussion

All clinicians and dentists, who deal with the oral cavity, dental infections, fractures, and other oral health issues, encounter various problems due to limited MO among children. Restriction in mandibular mobility is an indicator of mandibular dysfunction that further determines the state of the masticatory system in a patient. Hence the measurement of MMO becomes a relevant research topic for clinical practice. The current research aimed to assess the MMO among Saudi Arabian children and identify its association with demographics and different facial types. Data was collected from a convenient sample of 555 children, ranging from 2 to 12 years of age, with a mean age of  $4.99 \pm 3.15$ .

In this study, the average MMO was between 25.3 mm and 71.7 mm, with the mean  $\pm$  SD of  $47.3 \pm 8.7$  mm. In a previous studies by Moosa et al.<sup>14</sup> and AlHammad et al.<sup>15</sup> the researchers found the mean  $\pm$  SD of MMO to be  $46.1 \pm 8.5$  mm and  $47.8 \pm 6.9$  mm, respectively, which was in accordance with the results of our study. Most of the other studies reported similar values of MMO<sup>5,16</sup>.

As per the statistical analysis, it was found that the majority of the respondents (52.1%) had hyperleptoprosopic facial type. Fewer respondents (20%) had leptoprosopic facial type, and even fewer respondents (17.1%) had mesoprosopic facial type. A statistically significant difference was found between MMOs when compared based on facial profile; the mean MMO was higher in children with a concave facial profile as compared to those with a convex facial profile. Considering the relation between MMO and facial type, the results showed that MMO was higher among respondents with hyperleptoprosopic facial type. Compared with other facial types, the mean difference of MMO of subjects having mesoprosopic facial type was significantly lower than leptoprosopic and hyperleptoprosopic types. That is a novel contribution of the research since there are a few pieces of research in the previous literature that have determined the association or correlation between MMO and different facial types. Some studies, such as the study by Fukui et al.<sup>17</sup>, found a significant relationship between MMO and the facial types of female participants. Contrary to our findings, Fatima et al.<sup>5</sup> observed that MMO was higher among children with leptoprosopic facial type; furthermore, the researchers determined that hyperleptoprosopic facial type had a lower MMO value.

Concerning gender, 82.9% of male subjects and 37.9% of female subjects were found to have hyperleptoprosopic facial types. Leptoprosopic facial type was the second most common: 10.9% of male children and 24.2% of female children had this facial type. The facial indices of males were consistently higher than those of their female counterparts at various ages, but differences were statistically significant at 2, 3, 7, 8, and 9 years of age. The male subjects had hyperleptoprosopic facial type, while the female subjects predominantly had leptoprosopic type, except those at 3, 6, 11, and 12 years of age.

As for the current study analysis, a statistically non-significant difference was found when the MMO of chil-

dren was compared based on TMJ functioning. A few investigations have found that restricted mouth opening is usually associated with TMJ dysfunction syndrome<sup>18</sup>. This observation was in contrast with the findings of our research.

In the current study, the mean MMO was higher in female subjects than in male subjects. However, the difference in mean MMO was statistically significant only at 2, 3, 6, 7, 8, and 9 years of age when compared based on gender. This finding converges with a few previously reported studies<sup>19</sup> that have revealed higher mean MMO values among female subjects. Hirsch et al.<sup>20</sup> conducted longitudinal research and observed that female children and adults had higher MMO values, while male participants had lower values of MMO.

Several contradictory studies were found in this context. A study by Sridhar and Jeevanandham<sup>21</sup> investigated the association of MMO with age, gender, height, weight, and facial type among pediatric patients. The study found a concrete correlation between MMO and gender, where male subjects were found to have higher MMO values than females. A few other studies by Nagi et al.<sup>22</sup> and Patel et al.<sup>23</sup> reported similar deductions. Moosa et al.<sup>14</sup> also observed that male participants had higher MMO than females. Rashika and Gurunathan<sup>24</sup> observed that male children had a greater MMO than female children. AlHammad et al.<sup>15</sup> investigated the correlation between MMO, BMI, age, gender, and TMJ disorders in Saudi people. The researchers found that the mean MMO values of males were significantly greater than that of female participants. Al-Dlaigan and Asiry<sup>25</sup> revealed a statistically significant difference between the mouth opening of males and females. According to Fatima et al.<sup>5</sup>, there was a significant difference in MMO as per gender. The researchers also observed that MMO increases with age. On the contrary, Kumar et al.<sup>26</sup> found no gender differences concerning MMO. The study, however, revealed a significant association of MMO concerning age, height, and body weight. The variation in the findings of these studies might be attributed to the difference in sample size, study setting, population, study design, and methodology.

In our study, the hyperleptoprosopic type of face was most common among children, especially those with normal weight and overweight. The other facial types were seen in a higher proportion among normal weight and overweight than among underweight and obese children. The straight facial profile was observed more often in obese children than in other groups. The concave facial profile was seen in higher proportions among underweight children, while the convex facial profile was exhibited more by normal-weight children.

Our study found that MMO decreases with an increase in the weight of the respondents. Moreover, the analysis pointed out a significant positive correlation between MMO and facial type with age. A negative correlation was observed between MMO and facial type with the BMI of the children. Moreover, MMO was observed to decrease with an increase in BMI and the facial width of the chil-

dren. This observation was contradictory to the reports from the research conducted by Rashika and Gurunathan<sup>24</sup>, where children with higher weight had a greater MMO than the other children. AlHammad et al.<sup>15</sup> also determined a significant but weakly positive correlation of MMO with height and weight.

In the current study, mean MMO was shown to increase with age. Similarly, Sridhar and Jeevanandham<sup>21</sup> also revealed through their study that MMO increased with age. Rashika and Gurunathan<sup>24</sup> revealed a positive correlation between MMO, age, height, and body weight. The study reported a gradual increase of MMO in different age groups of the subjects. Koruyucu et al.<sup>27</sup> found no statistically significant difference in mean MMO values based on the gender of the subjects. However, the study found a positive association concerning age. Al-Dlaigan and Asiry<sup>25</sup> determined that regardless of gender, MMO increases significantly with age from the age of 12 to the age of 14 for the subjects included in their study. However, in contrast to our results, Moosa et al.<sup>14</sup> found that mean MMO values reduced with the age of the subjects.

#### Limitations of the study

A larger sample size could have revealed a more conclusive association of different parameters with MMO. Different ethnic groups could be studied in order to increase the external validity of the study outcome.

#### Conclusion

Within the limitations of the study, it was found that the overall mean value of MMO among the subjects was  $47.3 \pm 8.7$  mm. The MMO of subjects was found to have an association with different facial types and facial profiles among the studied population.

#### Funding

None.

#### Conflict of Interest

The authors declare no conflict of interest.

### R E F E R E N C E S

1. Lehman H, Fleissig Y, Abid-el-raziq D, Nitzan DW. Limited mouth opening of unknown cause cured by diagnostic coronoidectomy: A new clinical entity? *Br J Oral Maxillofac Surg* 2015; 53(3): 230–4.
2. Al-Dlaigan YH, Asiry MA. Maximum mouth opening in Saudi adolescents. *J Int Oral Health* 2014; 6(6): 45–9.
3. Zanawi KH, Al-Badawi EA, Lobo SL, Melis M, Mehta NR. An index for the measurement of normal maximum mouth opening. *J Can Dent Assoc* 2003; 69(11): 737–41.
4. Kumar A, Dutta S, Singh J, Mehta R, Hooda A, Namdev R. Clinical measurement of maximal mouth opening in children: A pioneer method. *J Clin Pediatr Dent* 2012; 37(2): 171–5.
5. Fatima J, Kaul R, Jain P, Saba S, Halder S, Sarkar S. Clinical measurement of maximum mouth opening in children of Kolkata and its relation with different facial types. *J Clin Diagn Res* 2016; 10(8): ZC01–5.
6. Li XY, Jia C, Zhang ZC. The normal range of maximum mouth opening and its correlation with height or weight in the young adult Chinese population. *J Dent Sci* 2017; 12(1): 56–9.
7. Forster CM, Sunga E, Chung CH. Relationship between dental arch width and vertical facial morphology in untreated adults. *Eur J Orthod* 2008; 30(3): 288–94.
8. Satiroglu F, Arun T, Isik F. Comparative data on facial morphology and muscle thickness using ultrasonography. *Eur J Orthod* 2005; 27(6): 562–7.
9. Kumari KL, Babu PVS, Kumari PK, Nagamani M. A study of cephalic index and facial index in Visakhapatnam, Andhra Pradesh, India. *Int J Res Med Sci*. 2015; 3(3): 656–8.
10. Yesmin T, Thwin SS, Urmi SA, Wai MM, Zaini PU, Azwan K. A study of facial index among Malay population. *J Anthropol* 2014; 2014: doi: <http://dx.doi.org/10.1155/2014/726974>.
11. Agrawal J, Shenai PK, Chatra L, Kumar PY. Evaluation of normal range of mouth opening using three finger index: South India perspective study. *Indian J Dent Res* 2015; 26(4): 361–5.
12. Obinna RO, Emmanuel NO, Johnson UA. Facial index among Igbo children and adolescents in Enugu. *Biomed Res* 2019; 30(6): 845–9.
13. Banabilh SM, Samsudin AR, Suzina AH, Dinsubaimi S. Facial profile shape, malocclusion and palatal morphology in Malay obstructive sleep apnea patients. *Angle Orthod* 2010; 80(1): 37–42.
14. Moosa ZH, Slibem AG, Junaidallah AA, Alshabtri AA, Al Samb AK, Kandil MM. Maximum mouth opening and its association with gender, age, height, weight, body mass index, and systemic disease in adult Saudi population: A cross-sectional study. *J Int Oral Health* 2020; 12(2): 173–81.
15. AlHammad ZA, Alomar AF, Alshammeri TA, Qadoumi MA. Maximum mouth opening and its correlation with gender, age, height, weight, body mass index, and temporomandibular joint disorders in a Saudi population. *Cranio* 2021; 39(4): 303–9.
16. Prasad M, Hussain MZ, Shetty SK, Kumar TA, Khaur M, George SA, et al. Median mandibular flexure at different mouth opening and its relation to different facial types: A prospective clinical study. *J Nat Sci Biol Med* 2013; 4(2): 426–30.
17. Fukuji T, Tsuruta M, Murata K, Wakimoto Y, Tokiwa H, Kuwabara Y. Correlation between facial morphology, mouth opening ability, and condylar movement during opening–closing jaw movements in female adults with normal occlusion. *Eur J Orthod* 2002; 24(4): 327–36.
18. Gallagher C, Gallagher V, Whelton H, Cronin M. The normal range of mouth opening in an Irish population. *J Oral Rehabil* 2004; 31(2): 110–6.
19. Pullinger AG, Liu SP, Low G, Tay D. Differences between sexes in maximum jaw opening when corrected to body size. *J Oral Rehabil* 1987; 14(3): 291–9.
20. Hirsch C, John MT, Lautenschläger C, List T. Mandibular jaw movement capacity in 10-17-yr-old children and adolescents: normative values and the influence of gender, age, and temporomandibular disorders. *Eur J Oral Sci* 2006; 114(6): 465–70.
21. Sridhar M, Jeevanandham G. Clinical measurement of maximum mouth opening in children and its relation with different facial types. *Drug Invent Today* 2018; 10(2): 3069–73.
22. Nagi R, Sabu S, Galwai D, Jain S. Study on evaluation of normal range of maximum mouth opening among Indian adults using three finger index: A descriptive study. *J Indian Acad Oral Med Radiol* 2017; 29(3): 186–90.

23. Patel SM, Patel NH, Khaïtan GG, Thanvi RS, Patel P, Joshi RN. Evaluation of maximal mouth opening for healthy Indian children: percentiles and impact of age, gender, and height. *Natl J Maxillofac Surg* 2016; 7(1): 33–8.
24. Rashika V, Gurumathan D. Clinical Measurement of Maximal Mouth Opening in Children of Age from 3 To 12 Years in Chennai-A Cross Sectional Study. *Res J Pharm Technol* 2018; 11(3): 1092–6.
25. Al-Dlaigan YH, Asiry MA. Maximum mouth opening in saudi adolescents. *Journal of international oral health: J Int Oral Health* 2014; 6(6): 45–9.
26. Kumar A, Mehta R, Goel M, Dutta S, Hooda A. Maximal mouth opening in Indian children using a new method. *J Cranio Max Dis* 2012; 1(2): 79–86.
27. Koryucu M, Tabakçilar D, Seymen F, Gençay K. Maximum mouth opening in healthy children and adolescents in Istanbul. *Dentistry 3000* 2018; 6(1): 1–7.

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## Expression of Kv4.2 and Kv4.3 potassium channels in human umbilical veins from normal, diabetic, and hypertensive pregnancies

Ekspresija kalijumovih kanala Kv4.2 i Kv4.3 u humanim pupčanim venama zdravih trudnica, trudnica sa gestacijskim dijabetesom melitusom i trudnica sa gestacijskom hipertenzijom

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### Abstract

**Background/Aim.** A substantial line of evidence indicates that Kv4.2 and Kv4.3 channels are the major components of rapid transient-outward potassium currents (A-type currents). It is speculated that those currents may be involved in the maintenance of the membrane potential, as well as in the regulation of propagation and frequency of action potentials. However, very little is known about the presence and function of A-type currents in human vascular smooth muscles such as the human umbilical vein (HUV). Bearing in mind its crucial role in the proper fetal oxygenation, the aim of the study was to determine whether Kv4.2 and Kv4.3 potassium channels are present in HUV smooth muscle and to investigate potential alterations of their expression during maternal pathological conditions such as gestational diabetes mellitus (GDM) and pregnancy-induced hypertension (PIH). **Methods.** Healthy, diabetic, and hypertensive pregnancies were subjects of this investigation. Each group consisted of 6 HUV samples obtained from 6 normal

pregnancies, 6 pregnancies with GDM, and 6 with PIH. After pharmacology analysis, immunohistochemistry (IH) and Western blot were performed. **Results.** IH revealed similar expression patterns of both, Kv4.2 and Kv4.3 subunits in HUV smooth muscle in all groups of patients. Results obtained by Western blot were in agreement with IH staining. The expression of Kv4.2 and Kv4.3 subunits were not significantly different between the groups. **Conclusion.** Collectively, this is the first study that demonstrated the presence of Kv4.2 and Kv4.3 potassium channels in the HUV smooth muscle and their preservation during GDM and PIH pregnancies. These channels are most likely major components of rapid A-type currents that may be relevant for maternal-fetus blood flow and hence fetal development. In addition, they may represent sensors for detecting hemodynamic and/or metabolic changes in the local environment.

**Key words:** diabetes mellitus; humans; hypertension; pregnancy; umbilical veins.

### Apstrakt

**Uvod/Cilj.** Značajan niz dokaza ukazuje na to da su kanali Kv4.2 i Kv4.3 glavne komponente brzih prolazno-ispravljačkih struja kalijuma (struje tipa A). Pretpostavlja se da bi te struje mogle da budu uključene u održavanje membranskog potencijala ćelije, kao i u regulaciju propagacije i učestalosti akcionih potencijala. Međutim, vrlo malo se zna o prisustvu i funkciji struje tipa A u glatkim mišićima krvnih sudova čoveka, kao što je humana umbilikalna vena (HUV). S obzirom na njenu ključnu ulogu u adekvatnoj oksigenaciji fetusa, cilj rada bio je da se utvrdi da li su podtipovi

kalijumovih kanala Kv4.2 i Kv4.3 prisutni u glatkim mišićima HUV-a i da se istraže potencijalne promene njihove ekspresije tokom patoloških stanja majke – gestacijskog dijabetesa melitusa (GDM) i arterijske hipertenzije (AH) izazvane trudnoćom (AHT). **Metode.** U istraživanje su uključene HUV sakupljene posle trudnoće zdravih porodilja, onih čije trudnoće su bile komplikovane GDM i AH. Svaka grupa sastojala se od 6 uzoraka HUV-a dobijenih iz 6 zdravih trudnoća, 6 trudnoća komplikovanih GDM i 6 trudnoća komplikovanih AH. Nakon farmakoloških analiza, urađene su imunohistoheksijska (IH) analiza i *Western blot*. **Rezultati.** Primenom IH analize pokazan je sličan obrazac ekspresije



obe podjedinice Kv4.2 i Kv4.3 kanala u glatkim mišićima HUV-a u svim grupama trudnica. Rezultati dobijeni *Western blot*-om bili su u skladu sa IH bojenjem. Ekspresija podjedinica Kv4.2 i Kv4.3 nije se značajno razlikovala između grupa trudnica. **Zaključak.** Ovo je prema našim saznanjima prva studija kojom je pokazano prisustvo Kv4.2 i Kv4.3 kalijumovih kanala u glatkim mišićima HUV-a zdravih porodilja, kao i onih porodilja čija je trudnoća bila praćena prisustvom GDM ili AHT. Ti kanali su najverovatnije glavne

komponente brzih struja A-tipa, koji mogu biti relevantni za protok krvi majke i ploda i na taj način delovati protektivno za razvoj fetusa. Takođe, oni mogu predstavljati senzitivne pokazatelje hemodinamskih i/ili metaboličkih promena u lokalnoj fetomaternalnoj sredini.

**Ključne reči:**  
dijabetes melitus; ljudi; hipertenzija; trudnoća; v.umbilicalis.

## Introduction

The connection between a mother and a child represents the most valuable nature relationship that begins *in utero* and lasts forever. During the antenatal period, the umbilical cord represents an important organ, as it maintains the functionality and integrity of a fetomaternal unit. It has a specific structure – two human umbilical arteries embedded in mucous connective tissue with one human umbilical vein (HUV) <sup>1</sup>. Mucous connective tissue (Wharton's jelly) gives the umbilical cord remarkable elasticity and represents its important feature <sup>2</sup> considering that this structural specificity allows proper fetal supplementation of nutrients and gas. The additional feature of the umbilical cord is represented by the absence of lymphatics, *vasa vasorum*, and innervations, which defines overall umbilical circulation as unique. The properties of the so-called low resistance – low-pressure umbilical circulation lead to amplified effects of systemic vasoactive substances and local inflammatory and mechanical factors in regulating umbilical blood vessel tone <sup>3</sup>.

The level of adequate oxygenation and nutritive supply is influenced by the undisturbed blood flow through the umbilical cord <sup>4</sup>. Furthermore, there are several important factors known to have an impact on placental blood flow, including fetal respiration, smooth muscle cell (SMC) contraction, and different pressure gradients caused by the contractility of a fetal heart. Another important factor for the quality of proper supply is the preservation of propagation direction of the umbilical arteries <sup>4, 5</sup> and exhibitions of HUVs autoregulatory vasomotor phenomena, which are additional factors in maintaining the blood flow <sup>6</sup>. The capability of HUVs to regulate their tone is an important fact, considering various pathological conditions that can affect the proper development of the fetus. For instance, hypoxia leads to the activation of the HUVs regulatory mechanisms and consecutive vasodilatation, so it can be concluded that HUV is not just a passive conduit blood vessel <sup>7</sup> and can regulate its diameter depending on the environmental settings. Histologically, vascular SMCs within the *tunica media* of the HUV consist of three types of differently oriented layers: circular, oblique, and longitudinal. The property of those layers, together with the compact structure of the Wharton's jelly, contributes to the maintenance of adequate and undisturbed blood flow through the umbilical cord, which is especially important during fetal movements <sup>8</sup>.

In 1949, the squid giant axon served as an animal model for Hodgkin and Huxley to report a discovery – electrical

currents through Kv channels. After that, around three decades had passed before that statement on vascular SMCs was confirmed <sup>9</sup>. Now it is known that vascular SMCs contain Kv currents that can be divided into two main types, regarding their time-dependency property: slow “delayed-rectifier” and rapid “transient-outward” K<sup>+</sup> currents (also known as A-type currents) <sup>10, 11</sup>. As for delayed-rectifier K<sup>+</sup> current, it shows delayed activation and slow inactivation and has been detected in the majority of vascular SMCs. Furthermore, those currents are responsible for the repolarization and setting of the resting membrane potential. The threshold for the activation in SMCs is about -30 mV <sup>12-14</sup>. On the contrary, A-type K<sup>+</sup> currents show very rapid activation, as well as inactivation. This current is less frequently detected in vascular SMCs; it has a relatively small contribution to the total outward Kv currents and is mainly inactivated at the resting membrane potential. A-type currents are activated at negative (sub-threshold) membrane potentials, typically between -45 and -60 mV. Additional properties typical for these types of currents are inactivation caused by voltage-dependence, strong steady state, and repolarization to the potentials negative to -50 mV, typically required for regeneration of channel function <sup>15</sup>. Since the discovery of A-type of K<sup>+</sup> currents in the human SMCs, detailed studies that have been examining its function and presence in human HUVs are limited <sup>16</sup>. In recent years, studies that have been using molecular techniques of Kv proteins in other tissues have demonstrated that the Kv4 subfamily of Kv channels (Kv4.1, Kv4.2, and Kv4.3) contributes to the overall A-type currents. Moreover, newer studies provide a substantial line of pieces of evidence specifically indicating that particularly Kv4.2 and Kv4.3 channels are the major components of A-type currents in many SMC, including vascular SMCs <sup>15-17</sup>.

The aim of this study was to reveal whether Kv4.2 and Kv4.3 potassium channels are present in the SMCs of HUV and to investigate potential alterations of their expression during pathological conditions such as gestational diabetes mellitus (GDM) and pregnancy-induced hypertension (PIH) that can alter blood flow to the fetus.

## Methods

### Ethics statement

The permission to conduct this research was obtained from the Ethics Committee of the Faculty of Medicine,

University of Belgrade, Serbia (permission No 2650/VI-5, from June 26, 2018).

#### *Collecting of the tissues and their manipulation*

Samples of umbilical cords were collected from the Clinic for Gynecology and Obstetrics "Narodni front", Belgrade, Republic of Serbia. All women have agreed to participate in the study by signing the written consent. The objects of investigation (umbilical cords) were collected immediately after vaginal delivery or Caesarean section, no later than 2 hrs after labor. Afterward, they were transported to the laboratory in small vials filled with cold Krebs-Ringer bicarbonate<sup>18</sup>. Samples were divided into three groups based on the health status of a pregnant woman: "normal" (healthy, i.e., having no known or apparent active disease) and two "experimental" groups: diabetic and hypertensive. After the preparation, HUVs were immersed in fixative (10% formaldehyde) for immunohistochemical staining and frozen at -70 °C for the Western blot analysis.

#### *Clinical terms and definitions*

GDM was implied as diabetes mellitus developed or recognized during pregnancy<sup>2</sup>.

Elevated blood pressure developed after 20 weeks of gestation with sustained values of systolic blood pressure  $\geq 140$  mmHg and/or diastolic blood pressure  $\geq 90$  mmHg was termed PIH. It is a wide entity and includes the following disorders: gestational hypertension, preeclampsia, and eclampsia<sup>2, 19</sup>.

#### *Immunohistochemistry*

The procedures of molecular technique and staining methods used in this study after collecting and preparation of the samples have been published earlier<sup>5, 20</sup>. In short, after the isolation from the umbilical cords, HUV samples were fixed in 10% formaldehyde and embedded in paraffin. All sections were deparaffinized and rehydrated through solutions with decreasing alcohol concentrations to distilled water and heated for 30 min in Tris-ethylenediaminetetraacetic acid (EDTA) buffer at pH 9.0 for antigen retrieval. Sections were washed with Tris-buffered saline and incubated overnight with anti-Kv4.2 and anti-Kv4.3 goat polyclonal primary antibodies (Santa Cruz Biotechnology, Inc., dilution ratio 1 : 50). Then they were treated by applying the commercial ImmunoCruz™ goat ABC Staining System (sc-2023, Santa Cruz Biotechnology). Diaminobenzidine as chromogen served as a developing tool for immunoreactions for 10 min.

**Table 1**

#### **Scoring system of Kv channel subunit for immunocytochemical analysis (adapted from Adams et al.<sup>22</sup>)**

Strong	More than 50% of cells are involved. Black/opaque staining that is clearly visible.
Intermediate	Less than 50% of cells are involved (with dark/opaque staining that is clearly visible) or more than 50% of cells (with intermediate membrane staining).
Weak	More than 50% of cells are involved (with intermediate focal staining that is clearly visible) or an unidentified percent of cells with light membrane appearance.
Sporadic	Unidentified percent of randomly distributed cells (with black/opaque membrane staining).
None	This group is represented by smooth muscle cells that show none of the aforementioned.

More detailed procedures can be found in the paper by Djokic et al.<sup>5</sup>.

The experienced researcher evaluated the intensity and the distribution of positive staining using two scoring systems. The scoring system for immunocytochemical analysis used in this paper was initially introduced by Fisher et al.<sup>21</sup> and was later adapted by Adams et al.<sup>22</sup>.

This scoring system unites the intensity and distribution of positive staining into one score and is shown in Table 1<sup>22</sup>. The other scoring system was a semi-quantitative four-point scale that scored the intensity of slides as negative and positive (+, ++, or +++)<sup>21, 22</sup>.

#### *Western blot analysis*

HUV rings were homogenized on ice in modified RIPA buffer (50 mmol/L Tris/HCl, pH 7.4, 150 mmol/L NaCl, 1% Triton X-100, 0.2% Na-deoxycholate, 0.2% SDS, 1 mmol/L EDTA, and protease and phosphatase inhibitors). The homogenates were centrifuged at  $15,000 \times g$  for 30 min at +4 °C. The BCA method was performed to determine the concentration of proteins. Obtained supernatants represented a cell lysate for Western blot after being boiled in the Laemmli sample buffer. The proteins were separated by electrophoresis on a 10% polyacrylamide gel and moved onto Polyvinylidene difluoride membranes. The type of membrane, blockers signal detecting, and visualization techniques are described in the author's previous paper<sup>23</sup>.

#### *Statistical analysis*

Data analysis was conducted with SigmaPlot (Systat Software Inc., San Jose, CA). The results are presented as the means  $\pm$  standard error of the mean (S.E.M.); n = number of experiments. The Student's *t*-test was used to determine the significance of differences for Western blot. After statistical analysis, *p*-values  $< 0.05$  were considered statistically significant.

#### **Results**

Each group consisted of 6 HUV samples obtained from 6 normal pregnancies, 6 pregnancies with GDM, and 6 pregnancies with PIH.

#### *Immunohistochemistry*

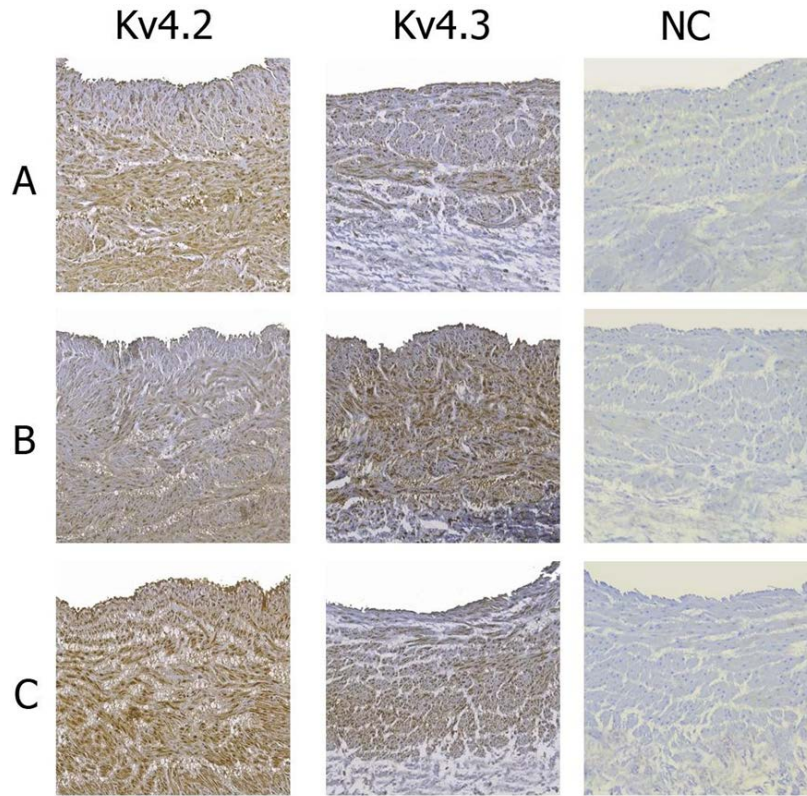
Immunohistochemistry analysis indicated membranous and cytoplasmic expression of Kv4.2 and Kv4.3 subunits in SMCs of HUVs. The expression of Kv4.2

showed a similar staining pattern in GDM and PIH compared to normal pregnancy (immunocytochemical scoring – moderate/++). The expression of the Kv4.3 subunit was similar through *tunica media* in all HUVs regardless of the sample group: normal pregnancies, GDM, or PIH. The expression pattern was also similar in all experimental groups (immunocytochemical scoring – moderate/++) (Figures 1A-1C).

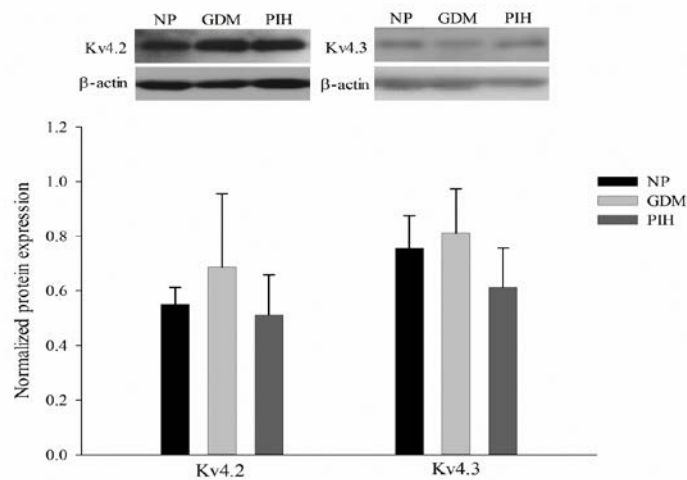
*Western blot analysis*

The Western blot results were in agreement with immunohistochemical staining. There were no detected differences in the expression of Kv4.2 and Kv4.3 subunits between the groups (Figure 2).

In the end, all results referring to Kv4.2 and Kv4.3 subunits distribution and expression are shown in Table 2.



**Fig. 1 – Immunohistochemical staining of Kv4.2 and Kv4.3 subunits. Normal pregnancy (A); gestational diabetes mellitus (B); pregnancy-induced hypertension (C). Expression of Kv4.2 and Kv4.3 (brown staining); negative control (NC). Original magnification: 200x. The figure is representative of the preparations of four patients.**



**Fig. 2 – The expression of Kv4.2 and Kv4.3 subunits on human umbilical veins from normal pregnancy (NP, n = 6), gestational diabetes mellitus (GDM, n = 6), and pregnancy-induced hypertension (PIH, n = 6). Results are expressed as mean ± standard error of the mean.**

Table 2

**Distribution and expression of Kv channel subunits according to immunohistochemistry (IH) and Western blot (WB)**

Subunit	NP (IH)	GDM (IH)	PIH (IH)	WB
Kv4.2	moderate/++	moderate/++	moderate/++	n.s.
	m/cp, diffuse	m/cp, diffuse	m/cp, diffuse	
Kv4.3	moderate/++	moderate/++	moderate/++	n.s.
	m/cp, diffuse	m/cp, diffuse	m/cp, diffuse	

NP – normal pregnancy; GDM – gestational diabetes mellitus; PIH – pregnancy induced hypertension; m/cp – membranous/cytoplasmic, n.s. – no statistically significant difference.

### Discussion

Based on our information so far, this is the first study to evaluate the expression of Kv4.2 and Kv4.3 channels in the HUV SMC obtained from healthy, diabetic, and hypertensive pregnancies. The principal new findings show that Kv4.2 and Kv4.3 channels are expressed in all experimental groups. The expression of Kv4.2 and Kv4.3 subunits remains preserved in both pathological conditions in comparison to normal pregnancy. The distribution of both channel subtypes appeared similar in HUV SMC from all groups. Activating voltage-gated potassium (Kv) channels hyperpolarizes and relaxes vascular SMCs by decreasing the activity of L-type voltage-gated Ca<sup>2+</sup> channels. Conversely, inhibition of Kv channels induces vasoconstriction<sup>24</sup>. Vascular SMCs have been reported to express members of the Kv1, Kv2, Kv3, Kv4, Kv6, Kv7, Kv9, and Kv11 subfamilies of Kv channels<sup>25</sup>.

Kv channels are complexes of several subunits constructed of membrane-integrated  $\alpha$ -subunits and accessory subunits. The  $\alpha$ -subunits form a tetrameric complex that represents the core of the Kv channel, which consists of a pore domain with activation/inactivation gates and a selectivity filter connected to peripheral voltage-sensor domains. Auxiliary subunits may increase the diversity of Kv channels by changing the function and expression of the  $\alpha$ -subunits and control membrane trafficking<sup>14,26</sup>.

HUV responds very actively to different *stimuli*, especially to ones expected to be abnormal, often seen in diabetic and hypertensive pregnancies. Previously, we have shown that the ability of pinacidil (recognized as a potassium channel opener) to produce vasorelaxation of HUVs from healthy pregnancies in the presence of nonspecific Kv1-4 channels inhibitor, 4-aminopyridine (4-AP), is decreased<sup>26</sup>. Compared to HUVs obtained from GDM and PIH, these effects were absent<sup>5</sup>. Furthermore, in order to obtain more details about the Kv channel subtypes involved in the relaxation produced by pinacidil on HUVs, we have used margatoxin (MgTx), a specific blocker of Kv1.2 and Kv1.3 channels. In the presence of MgTx, pinacidil-induced vasorelaxation of HUVs from normal pregnancies was significantly reduced, confirming the involvement of Kv1.2 and/or Kv1.3 in the pinacidil response<sup>27</sup>.

These findings made us conduct additional experiments in order to check the presence of Kv4.2 and Kv4.3 channels in HUV SMC, bearing in mind that the inhibition by 4-AP is

considered one of the pharmacological hallmarks of A-type currents<sup>15</sup>. Our assumption was that their expression could be reduced in GDM and PIH.

A-type currents were first described in molluscan neurons<sup>28</sup>. It was suggested that they have roles in controlling neuronal action potential threshold, frequency, and duration<sup>17</sup>. A-type currents have also been detected in a myriad of non-neural tissues, including atrial and ventricular cardiomyocytes and visceral and vascular SMCs. In contrast to neuronal A-type currents, currents found in cardiomyocytes were available at resting membrane potentials and were predominantly responsible for the initial repolarization (phase 1) of the cardiac action potential. In SMCs, A-type currents may be related to the maintenance of membrane potential. In addition, they may regulate propagation and frequency of action potentials<sup>29,30</sup>.

It has been reported that A-type current coexists with the delayed-outward current in vascular SMCs of the rabbit (portal vein, pulmonary artery, aorta), rat (pulmonary artery, renal resistance artery), and human mesenteric artery. In all mentioned blood vessels, Kv4.2 and Kv4.3 were detected as major or particularly important components of A-type currents in vascular SMC<sup>12,15</sup>. For the first time, we detected in the present study the expression of Kv4.2 and Kv4.3 subunits throughout the *tunica media* of the HUVs from normal, diabetic, and hypertensive pregnancies. That confirmed the presence of A-type currents in the HUV SMC. Moreover, the differences in the expression of the aforementioned channels between the groups were not statistically significant, confirming that the expression of Kv4.2 and Kv4.3 subunits in diabetic and hypertensive veins remains preserved in pathological pregnancies. That, for instance, is in contrast with our previous research conducted on ATP-sensitive K channels, where reduced Kir6.1 expression in vascular SMCs of the HUV during GDM and PIH was found<sup>5</sup>. Observations that A-type currents in vascular SMCs often coexist with delayed-outward K<sup>+</sup> currents and that 4-AP is a nonspecific Kv1-4 inhibitor<sup>12,31</sup> lead to the conclusion that alterations of some other members of the Kv channel family are probably responsible for the loss of antagonism to 4-AP during GDM and PIH in our previous study. This statement will probably be the basis of our further research.

Diminished expression of Kv4.2 and Kv4.3 subunits have been reported in cardiac myocytes from spontaneously hypertensive rats alongside lower A-type currents density<sup>32,33</sup>. Moreover, downregulation of the Kv4.2, but not

Kv4.3 channels, have been reported in diabetic rat ventricle and human diabetic cardiomyopathy<sup>34, 35</sup>. However, by reviewing the literature, we have not found any research that has studied alterations of these channels in vascular SMC during diabetes mellitus and hypertension in other vascular beds and other species.

To date, the precise physiological role of A-type currents in vascular SMCs has not been completely clarified. Some reports have suggested that the primary role of the A-type current in vascular SMCs is to suppress membrane excitability. In retinal microvascular SMCs, the application of 4-AP to current-clamped vessels has caused membrane depolarization alongside increased cell contractility. Thus, it has been revealed that regulation of A-type currents in retinal vascular SMCs may be of great importance for the control of local tissue perfusion<sup>11</sup>. Bearing in mind that HUV is able to adjust its vascular tone influenced by various factors of the local microenvironment, we can speculate that A-type channels in HUV may be sensors for detecting hemodynamic and/or metabolic changes in the local environment. Furthermore, another possibility is that A-type currents play a role in the genesis of myogenic oscillations of the vein. Namely, in rabbit portal vein, it has been demonstrated that A-type currents tend to be a feature of phasic SMCs<sup>15</sup>. In HUV, these currents and hence Kv4.2 and Kv4.3 channels may be involved in the vasomotion phenomena. In that way, by producing myogenic oscillations, A-type currents might be significant for umbilical venous blood flow and may contribute to fetal development<sup>6</sup>.

#### Limitations of the study

A limitation of this research is that A-type currents were not measured and recorded using methods of electrophysiology or real-time polymerase chain reaction. However, it should be highlighted that, in all previous publications,

Kv4.2 and Kv4.3 channel subunits were, without exception, identified as major constituents of A-type currents.

#### Conclusion

To the best of our knowledge, this study was the first that demonstrated the presence of Kv4.2 and Kv4.3 potassium channels in the HUV SMCs and their preservation during the course of GDM and PIH. These channels are most likely major components of A-type currents that may be relevant for mother-fetus blood flow and hence fetal development. Additionally, they may represent sensors for detecting hemodynamic and/or metabolic changes in the local environment. Further investigations in this field may be of great importance for understanding the regulation of umbilical circulation and may provide new strategies for reducing the risk of negative pregnancy outcomes.

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#### Conflict of Interest

The authors declare no conflict of interest.

## R E F E R E N C E S

1. Moshiri M, Zaidi SF, Robinson TJ, Bhargava P, Siebert JR, Dubinsky TJ, et al. Comprehensive imaging review of abnormalities of the umbilical cord. *Radiographics* 2014; 34(1): 179–96.
2. Blanco MV, Vega HR, Guerrí-Guttenberg RA, Giulianob R, Granac DR, Azcatocet F, et al. Histopathology and histomorphometry of umbilical cord blood vessels. Findings in normal and high risk pregnancies. *Artery Res* 2011; 5(2): 50–7.
3. Wareing M. Oxygen sensitivity, potassium channels, and regulation of placental vascular tone. *Microcirculation* 2014; 21(1): 58–66.
4. Spurway J, Logan P, Pak S. The development, structure and blood flow within the umbilical cord with particular reference to the venous system. *Australas J Ultrasound Med* 2012; 15(3): 97–102.
5. Djokić V, Janković-Raznatović S, Novaković R, Kostić M, Rajković J, Labudović-Borović M, et al. Effect of gestational diabetes mellitus and pregnancy-induced hypertension on human umbilical vein smooth muscle k(atp) channels. *Exp Mol Pathol* 2019; 111: 104323.
6. García-Huidobro DN, García-Huidobro MT, Huidobro-Toro JPG. Vasomotion in human umbilical and placental veins: Role of gap junctions and intracellular calcium reservoirs in their synchronous propagation. *Placenta* 2007; 28(4): 328–38.
7. Mildenerberger E, Siegel G, Versmold HT. Oxygen-dependent regulation of membrane potential and vascular tone of human umbilical vein. *Am J Obstet Gynecol* 1999; 181(3): 696–700.
8. Koeb A, Ndungu B, Gichangi P. Structural changes in umbilical vessels in pregnancy induced hypertension. *Placenta* 2008; 29(2): 210–4.
9. Hille B. *Ion channels of excitable membranes*. Sunderland, MA: Sinauer Associates; 2001.
10. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* 1995; 268(4 Pt 1): C799–822.
11. McGabon MK, Dawicki JM, Scholfield CN, McGeown JG, Curtis TM. A-type potassium current in retinal arteriolar smooth muscle cells. *Invest Ophthalmol Vis Sci* 2005; 46(9): 3281–7.
12. Xu C, Lu Y, Tang G, Wang R. Expression of voltage-dependent k(+) channel genes in mesenteric artery smooth muscle cells. *Am J Physiol* 1999; 277(5): G1055–63.
13. Jackson WF. Ion channels and vascular tone. *Hypertension* 2000; 35(1 Pt 2): 173–8.

14. *Pongs O, Schwarz JR.* Ancillary subunits associated with voltage-dependent k<sup>+</sup> channels. *Physiol Rev* 2010; 90(2): 755–96.
15. *Amberg GC, Koh SD, Imaizumi Y, Ohya S, Sanders KM.* A-type potassium currents in smooth muscle. *Am J Physiol Cell Physiol* 2003; 284(3): C583–95.
16. *Cai SQ, Li W, Sesti F.* Multiple modes of a-type potassium current regulation. *Curr Pharm Des* 2007; 13(31): 3178–84.
17. *Ohya S, Tanaka M, Oku T, Asai Y, Watanabe M, Giles WR, et al.* Molecular cloning and tissue distribution of an alternatively spliced variant of an a-type k<sup>+</sup> channel alpha-subunit, kv4.3 in the rat. *FEBS Lett* 1997; 420(1): 47–53.
18. *Radenković M, Grbović L, Radunović N, Momčilov P.* Pharmacological evaluation of bradykinin effect on human umbilical artery in normal, hypertensive and diabetic pregnancy. *Pharmacol Rep* 2007; 59(1): 64–73.
19. *Yang CC, Tang PL, Liu PY, Huang WC, Chen YY, Wang HP, et al.* Maternal pregnancy-induced hypertension increases subsequent neonatal necrotizing enterocolitis risk: A nationwide population-based retrospective cohort study in Taiwan. *Medicine (Baltimore)* 2018; 97(31): e11739.
20. *Rakocevic J, Kojic S, Orlic D, Stankovic G, Ostojic M, Petrovic O, et al.* Co-expression of vascular and lymphatic endothelial cell markers on early endothelial cells present in aspirated coronary thrombi from patients with st-elevation myocardial infarction. *Exp Mol Pathol* 2016; 100(1): 31–8.
21. *Fisher CJ, Gillett CE, Vojtšek B, Barnes DM, Millis RR.* Problems with p53 immunohistochemical staining: The effect of fixation and variation in the methods of evaluation. *Br J Cancer* 1994; 69(1): 26–31.
22. *Adams EJ, Green JA, Clark AH, Youngson JH.* Comparison of different scoring systems for immunohistochemical staining. *J Clin Pathol* 1999; 52(1): 75–7.
23. *Bundalo M, Zivkovic M, Culafic T, Stojiljkovic M, Koricanac G, Stankovic A.* Oestradiol treatment counteracts the effect of fructose-rich diet on matrix metalloproteinase 9 expression and nfκb activation. *Folia Biol (Praha)* 2015; 61(6): 233–40.
24. *Nieves-Cintrón M, Syed AU, Nystoriak MA, Navedo MF.* Regulation of voltage-gated potassium channels in vascular smooth muscle during hypertension and metabolic disorders. *Microcirculation* 2018; 25(1): 10.1111/micc.12423..
25. *Jackson WF.* K(v) channels and the regulation of vascular smooth muscle tone. *Microcirculation* 2018; 25(1): 10.1111/micc.12421.
26. *Duzhy D, Harvey M, Sokolowski B.* A secretory-type protein, containing a pentraxin domain, interacts with an a-type k<sup>+</sup> channel. *J Biol Chem* 2005; 280(15): 15165–72.
27. *Djokic V, Jankovic S, Labudovic-Borovic M, Rakocevic J, Stanisic J, Rajkovic J, et al.* Pregnancy-induced hypertension decreases k(v)1.3 potassium channel expression and function in human umbilical vein smooth muscle. *Eur J Pharmacol* 2020; 882: 173281.
28. *Hagihara S, Kusano K, Saito N.* Membrane changes of onchidium nerve cell in potassium-rich media. *J Physiol* 1961; 155(3): 470–89.
29. *Amberg GC, Baker SA, Koh SD, Hatton WJ, Murray KJ, Horowitz B, et al.* Characterization of the a-type potassium current in murine gastric antrum. *J Physiol* 2002; 544(2): 417–28.
30. *Ohya S, Ito K, Hatano N, Ohno A, Muraki K, Imaizumi Y.* Castration induces down-regulation of a-type k(+) channel in rat vas deferens smooth muscle. *Int J Mol Sci* 2019; 20(17): 4073.
31. *Alexander SPH, Mathie A, Peters JA, Veale EL, Striessnig J, Kelly E, et al.* The concise guide to pharmacology 2019/20: Ion channels. *Br J Pharmacol* 2019; 176(Suppl 1): S142–S228.
32. *Goltz D, Schultze JH, Stucke C, Wagner M, Bassalaj P, Schnoerer AP, et al.* Diminished kv4.2/3 but not kchip2 levels reduce the cardiac transient outward k<sup>+</sup> current in spontaneously hypertensive rats. *Cardiovasc Res* 2007; 74(1): 85–95.
33. *Zhang H, Wu S, Huang C, Li X.* Long-term treatment of spontaneously hypertensive rats with losartan and molecular basis of modulating ito of ventricular myocytes. *Mol Med Rep* 2014; 9(5): 1959–67.
34. *Nishiyama A, Ishii DN, Backx PH, Pulford BE, Birks BR, Tamkun MM.* Altered k(+) channel gene expression in diabetic rat ventricle: Isoform switching between kv4.2 and kv1.4. *Am J Physiol Heart Circ Physiol* 2001; 281(4): H1800–7.
35. *Miki T, Yuda S, Kouzu H, Miura T.* Diabetic cardiomyopathy: Pathophysiology and clinical features. *Heart Fail Rev* 2013; 18(2): 149–66.

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## Decompression procedure as a successful initial approach to a large maxillary dentigerous cyst in a 12-year-old boy – a case report

Postupak dekompresije kao uspešan inicijalni pristup u lečenju velike maksilarne folikularne ciste kod 12-godišnjeg dečaka

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### Abstract

**Introduction.** A dentigerous cyst is a pathological lesion associated with the crown of the unerupted or impacted tooth. Decompression is proposed as the initial treatment for large cysts, especially in younger patients. The aim of this study was to present clinical, radiological, histopathological, and therapeutical aspects of the large dentigerous cyst in the maxilla. **Case report.** A 12-year-old boy with a large dentigerous cyst in the maxilla was referred to our clinic. A dentigerous cyst was associated with the crown of the unerupted maxillary left canine. Management of the cyst included initial decompression with biopsy, followed by secondary enucleation after 9 months and orthodontic treatment. Histopathological examination confirmed the definitive diagnosis of the dentigerous cyst. **Conclusion.** Initial decompression with a subsequent enucleation proved to be an effective treatment of choice for the large dentigerous cyst.

### Key words:

child; decompression, surgical; dentigerous cyst; diagnosis; maxilla; oral surgical procedures.

### Apstrakt

**Uvod.** Folikularna cista je patološka lezija, povezana sa krunicom neizniklih ili impaktiranih zuba. Dekompresija je predložena kao inicijalni tretman u terapiji velikih cista, posebno kod mladih pacijenata. Cilj rada bio je da se predstavljaju klinički, radiološki, patohistološki i terapijski aspekti velike folikularne ciste u maksili. **Prikaz bolesnika.** Dvanaestogodišnji dečak sa velikom folikularnom cistom u gornjoj vilici upućen je u našu kliniku. Folikularna cista se nalazila oko krunice neizniklog levog maksilarnog očnjaka. Terapija ciste obuhvatila je inicijalnu dekompresiju sa biopsijom, praćenu sekundarnom enukleacijom nakon 9 meseci, i ortodontski tretman. Histopatološka analiza potvrdila je konačnu dijagnozu folikularne ciste. **Zaključak.** Inicijalna dekompresija sa naknadnom enukleacijom pokazala se kao efikasna terapijska opcija u lečenju velike folikularne ciste.

### Ključne reči:

deca; dekompresija, hirurška; cista, odontogena; dijagnoza; maksila; hirurgija, oralna, procedure.

### Introduction

Dentigerous cysts are the second most prevalent odontogenic cysts (14–24% of all oral cysts), mainly located in the posterior parts of the mandible <sup>1</sup>. They often appear in the second and third decades of life, more commonly in males. In general, dentigerous cysts are asymptomatic in the early stages, but with the cyst progression, swelling, tooth mobility, and displacement might be clinically noticed <sup>2</sup>. Diagnosis of the dentigerous cysts is usually made

by radiographic examination and is confirmed by histopathological verification. Radiographically, they are presented as well-defined, unilocular lesions associated with the crown of the unerupted or impacted permanent teeth, most commonly mandibular third molars, maxillary canines, and maxillary third molars <sup>3</sup>. Histologically dentigerous cysts have connective tissue capsules permeated by young fibroblasts and sometimes odontogenic epithelial remnants, 2–3 layers thick epithelium with Rushton's bodies, filled with cystic fluid <sup>4</sup>.

Various treatment techniques have been described for managing cystic lesions, including decompression, marsupialization, enucleation, bone resection, and their combination<sup>5</sup>. Established criteria for the election of the most suitable therapeutic approach are cystic lesion dimensions and localization, histological presentation, patient age, the position of the affected tooth, and its relation with the adjacent anatomical structures<sup>6</sup>.

Decompression represents a specific conservative method, first described in 1958, that consists of making a small opening in the lesion and inserting different devices such as polyethylene tubes or stents<sup>7</sup>. The aim of this procedure is to reduce intracystic pressure by constant drainage and induce new bone formation, which consequently leads to a substantial decrease in cyst size<sup>8</sup>. The preservation of important anatomical structures, such as the inferior alveolar nerve and maxillary sinus, may be considered the main benefit of decompression<sup>9</sup>. With great success, it is used in treating large cystic lesions in younger patients due to the high regenerative potential of their oral tissues.

The aim of this article was to present clinical, radiological, histopathological, and therapeutical aspects of the dentigerous cyst in the maxilla of a 12-year-old patient.

## Case report

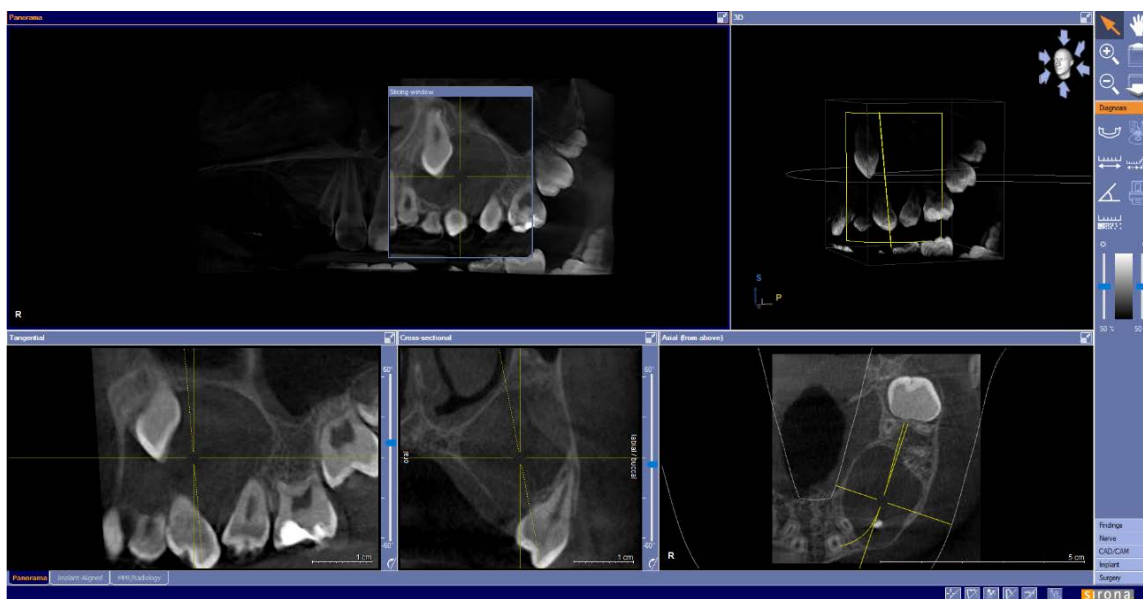
A 12-year-old male patient was referred to the Department of Dentistry of the Faculty of Medical Sciences in Kragujevac. He was in good general health, with no history of major medical conditions or syndromes. Written informed consent was obtained from the boy's parents for all procedures, imaging, and publication data.

The patient reported no pain or sensitivity during the first appointment. Intraoral examination indicated a healthy-looking mucosa with a slight, painless swelling in the area of the unerupted maxillary left canine on the buccal side. On palpation, fluctuation was present.

Orthopantomogram (OPG) revealed a large cyst located around the crown of the maxillary left canine, with well-defined borders (Figure 1). A complementary radiographic method, computed cone-beam tomography (CBCT), was used to assess the dimensions of the cyst more precisely and its relationship with surrounding structures (Figure 2). The rate of cyst reduction was monitored on panoramic radiographs taken after three, six, and nine months. When the size of the cyst was reduced enough to avoid adjacent structures,



**Fig. 1 – Panoramic radiography before therapy: a large cyst located around the crown of the maxillary left canine with well-defined borders.**



**Fig. 2 – Computed cone-beam tomography (CBCT) shows well-demarcated cystic lesions.**



surgical enucleation of the cyst was performed using the last panoramic that was taken.

The treatment plan was made according to the characteristics of the lesion and included initial decompression followed by secondary enucleation after 9 months. In order to facilitate the eruption of the involved teeth, orthodontic treatment was advised.

Decompression and biopsy of the cystic lesion were conducted with local anesthesia (4% articaine with 1 : 100,000 adrenaline; Septanest®, Septodont, France). The mucoperiosteal flap was elevated, after which one part of the cyst was excised and sent for histopathological verification. A tube was inserted in the cystic cavity to ensure proper drainage and regular saline irrigation in the following period. No signs of infection were recorded during control appointments in the next 9 months.

Histological examination revealed fibrous connective tissue lined with stratified, nonkeratinized squamous epithelium, which verified the diagnosis of the dentigerous cyst. Therefore, histopathological analysis represents an essential asset for establishing an accurate diagnosis.

The surgical intervention was performed under local anesthesia. OPG revealed a significant decrease in the cyst volume and thickening of its walls (Figure 3). After the decompression procedure, CBCT was not performed due to radiation dose reduction. A mucoperiosteal flap was elevated, and minimal bone resection was performed. After that, cyst remnants were eliminated, along with the profuse saline irrigation, to prevent lesion recurrence. The mucoperiosteal flap was relaxed, and sutures were performed.

After the intervention, the patient was prescribed prophylactic antibiotic therapy (amoxicillin 250 mg every 8 hrs), which was continued for five days postoperatively, and analgetic (ibuprofen 200 mg every 8 hrs) for two days. He was also instructed to maintain proper oral hygiene. Sutures were removed on the 7th postoperative day. The surgical site showed no signs of infection and healed without complications.

## Discussion

Dentigerous cysts form due to fluid collection between the reduced enamel epithelium and the crown of the unerupt-

ed or impacted tooth<sup>10</sup>. They can be categorized into two main groups: developmental, predominantly affecting molars, and inflammatory, mostly involving mandibular premolars<sup>2</sup>. Dentigerous cysts are a very common finding in mixed dentition.

Treatment of large odontogenic cystic lesions remains without standardized protocol. Several different procedures are available for cyst management, such as decompression, marsupialization, enucleation, and novel techniques like laser incisions and fenestration<sup>11</sup>. The ultimate goal is to remove the lesion completely and to avoid complications during and after the procedure. Decompression represents a conservative approach to treating cysts. Compared to other methods, it is significantly less technically demanding, less invasive, and has a lower incidence of postoperative complications<sup>6, 12</sup>. It results in the shrinkage of the cyst volume, the preservation of the surrounding tissues, and the decreased rate of pathological fractures<sup>9</sup>.

We used decompression as an initial method to treat a large maxillary dentigerous cyst, which proved effective, as the cyst volume decreased by approximately 70% after 9 months (Raša Mladenović, created a CorelDraw VBA Macro for the analysis of the cyst area, based on the difference between the first and last OPG). A remarkable percentage of the cyst shrinkage is due to the age of the patient and his high regenerative potential. Therefore, decompression may be a treatment of choice for the younger population. In a study that included 17 patients with cystic lesions of the jaw, the average reduction rate after decompression was 64%, with a mean decompression period of 8.1 months<sup>13</sup>. Oliveros-Lopez et al.<sup>14</sup> have shown that decompression as an initial treatment of odontogenic cysts is highly effective as it significantly reduces the size of the lesion without damaging surrounding structures. Riachi et al.<sup>15</sup> reported a remarkable increase in the bone formation and shrinkage of the dentigerous cyst of 48.5% in the 3 months following the decompression procedure. There are different opinions about the duration of the decompression, but various authors agree that a period from 6 to 14 months might be the most suitable period, which is in accordance with our report.



**Fig. 3 – Radiographic evaluation: panoramic view after decompression procedure.**

Diagnosis of dentigerous cysts includes clinical evaluation and radiographic imaging – OPG. However, in the cases of large lesions, a complementary radiographic method such as CBCT is necessary. It allows the three-dimensional assessment of the present pathological lesion and its relationship with adjacent anatomical structures. Moreover, CBCT enables precise measuring of the volumetric changes following decompression procedure<sup>8</sup>. These were the reasons why we chose this particular imaging method for our patient.

The main disadvantages of decompression are an extended time of healing, possible recurrence of the lesion, and patient cooperation. For this procedure to be successful, it is fundamental that patients are motivated enough to come for

checkups regularly during the follow-up period and maintain good oral hygiene. Our patient accepted all of the instructions and showed good compliance.

The subsequent surgical procedure of the cyst was performed without complications. Our case indicates that a decompression period of 9 months is sufficient for safe and successful enucleation.

### Conclusion

The decompression procedure may be regarded as a valuable therapeutic approach for managing large dentigerous cysts located in the vicinity of vital anatomical structures, especially in the younger population.

### R E F E R E N C E S

1. *Kirtaniya BC, Sachdev V, Singla A, Sharma AK.* Marsupialization: A conservative approach for treating dentigerous cyst in children in the mixed dentition. *J Indian Soc Pedod Prev Dent* 2010; 28(3): 203–8.
2. *Durmus B, Pekel B, Ugurlu F, Tanboga I.* Outcome of a dentigerous cyst following decompression using a removable appliance: a case report. *Oral Health Dent Manag* 2014; 13(1): 87–90.
3. *Meng Y, Zhao YN, Zhang YQ, Liu DG, Gao Y.* Three-dimensional radiographic features of ameloblastoma and cystic lesions in the maxilla. *Dentomaxillofac Radiol* 2019; 48(6): 20190066.
4. *Huang G, Moore L, Logan RM, Gue S.* Histological analysis of 41 dentigerous cysts in a paediatric population. *J Oral Pathol Med* 2019; 48(1): 74–8.
5. *Marin S, Kirnbauer B, Rugani P, Mellacher A, Payer M, Jakse N.* The effectiveness of decompression as initial treatment for jaw cysts: A 10-year retrospective study. *Med Oral Patol Oral Cir Bucal* 2019; 24(1): e47–e52.
6. *Yun SU, Jung HW, Cho BY, Choi BJ, Lee B, Kwon YD, et al.* Conservative Treatment Using Marsupialization for Cysts Occurring in the Jaw of Adolescents: A Case Report. *J Korean Dent Sci* 2014; 7(1): 31–7.
7. *Costa FW, Carvalho FS, Chaves FN, Soares EC.* A suitable device for cystic lesions close to the tooth-bearing areas of the jaws. *J Oral Maxillofac Surg* 2014; 72: 96–8.
8. *Kwon YJ, Ko KS, So BK, Kim DH, Jang HS, Kim SH, et al.* Effect of Decompression on Jaw Cystic Lesions Based on Three-Dimensional Volumetric Analysis. *Medicina (Kaunas)* 2020; 56(11): 602.
9. *Schlieve T, Miloro M, Kolokythas A.* Does decompression of odontogenic cysts and cystlike lesions change the histologic diagnosis? *J Oral Maxillofac Surg* 2014; 72(6): 1094–105.
10. *Chouchene F, Ameer WB, Hamdi H, Bouenba M, Masmoudi F, Baaziz A, et al.* Conservative Approach of a Dentigerous Cyst. *Case Rep Dent* 2021; 2021: 5514923.
11. *Scariot R, da Costa DJ, Rebellato NL, Müller PR, Gugisch RC.* Treatment of a large dentigerous cyst in a child. *J Dent Child (Chic)* 2011; 78(2): 111–4.
12. *AbouHosn M, Noujeim Z, Nader N, Berberi A.* Decompression and Enucleation of a Mandibular Radicular Cyst, Followed by Bone Regeneration and Implant-Supported Dental Restoration. *Case Rep Dent* 2019; 2019: 9584235.
13. *Lee ST, Kim SG, Moon SY, Oh JS, You JS, Kim JS.* The effect of decompression as treatment of the cysts in the jaws: retrospective analysis. *J Korean Assoc Oral Maxillofac Surg* 2017; 43(2): 83–7.
14. *Oliveros-Lopez L, Fernandez-Olavarria A, Torres-Lagares D, Serrera-Figallo MA, Castillo-Oyague R, Segura-Egea JJ, et al.* Reduction rate by decompression as a treatment of odontogenic cysts. *Med Oral Patol Oral Cir Bucal* 2017; 22(5): e643–e650.
15. *Riachi F, Khairallah CM, Ghosn N, Berberi AN.* Cyst volume changes measured with a 3D reconstruction after decompression of a mandibular dentigerous cyst with an impacted third molar. *Clin Pract* 2019; 9(1): 1132.

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## Bleeding hepatico-cutaneous fistula – an unusual complication of the percutaneous liver biopsy

Krvareća hepatično-kutana fistula – retka komplikacija perkutane biopsije jetre

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### Abstract

**Introduction.** An arteriovenous fistula is one of the complications that can occur during percutaneous liver biopsy (PLB). Hepatic arteriovenous fistula with chronic bleeding from the puncture site on the skin is an extremely rare complication following PLB. **Case report.** We present a 35-year-old woman with secondary anemia caused by chronic bleeding at the site of a granuloma caused by a previous liver biopsy performed 7 years ago. The patient was examined and treated for several years due to anemic syndrome. The pathological communication between the right hepatic vein, the anterior sectional branch of the portal vein, and the posterior arterial sectional branch were detected on a computed tomography scan and proven by fistulography. Due to the failed embolization, a laparotomy was performed, where a tumor mass was found in the VI and VII segment of the liver, which communicates with the skin. The tumor mass was removed by atypical resection of the VI and VII liver segments. Due to hemorrhage, reexploration was performed, where the bleeding was found from the surface of the resected liver parenchyma. The patient was released for home treatment two weeks after the last operation. **Conclusion.** Although PLB is a safe procedure, a complication in the form of bleeding sometimes occurs but with spontaneous cessation. In the presented patient, there was a complicated intrahepatic arteriovenous-portal fistula with the formation of communication with the puncture site on the skin. That is the first published case of complications of this type after PLB.

### Key words:

biopsy, fine-needle; diagnosis; hemorrhage; liver; surgical procedures, operative; vascular fistula.

### Apstrakt

**Uvod.** Arteriovenska fistula predstavlja jednu od komplikacija koja se može javiti prilikom perkutane biopsije jetre (PB). Hepatična arteriovenska fistula sa hroničnim krvarenjem iz mesta uboda na koži predstavlja izuzetno retku komplikaciju PB. **Prikaz bolesnika.** Prikazujemo 35-godišnju ženu sa sekundarnom anemijom izazvanom hroničnim krvarenjem na mestu granuloma, izazvanog prethodnom biopsijom jetre rađenoj 7 godina ranije. Bolesnica je nekoliko godina ispitivana i lečena zbog anemijskog sindroma. Patološka komunikacija između desne hepatične vene, prednje sekcijske grane vene porte i zadnje arterijske sekcijske grane je detektovana kompjuterizovanom tomografijom, a patološka vaskularizacija u desnom lobusu jetre je dokazana fistulografijom. Zbog neuspele embolizacije, učinjena je laparotomija i nađena je tumorska masa u VI i VII segmentu jetre, koja je komunicirala sa kožom. Tumorska masa je odstranjena atipičnom resekcijom VI i VII segmenta jetre. Zbog akutne hemoragije, posle dva dana učinjena je reeksploracija, kada je nađeno krvarenje sa površine reseciranog parenhima jetre. Bolesnica je otpuštena na kućno lečenje dve nedelje nakon poslednje operacije. **Zaključak.** Iako je PB sigurna procedura, nekada dolazi do komplikacije u vidu krvarenja koje spontano prestaje. Kod prikazane bolesnice je došlo do komplikovane intrahepatične arteriovensko-portne fistule sa formiranjem komunikacije sa mestom uboda na koži. To je prvi opisani slučaj komplikacije te vrste nastale posle PB.

### Ključne reči:

biopsija tankom iglom; dijagnoza; krvarenje; jetra; hirurgija, operativne procedure; fistula, vaskularna.

### Introduction

Liver biopsy is one of the most frequent diagnostic tools not only for liver pathologies but also for other systemic diseases. In particular, percutaneous liver biopsies

(PLB) constitute a large portion of all biopsies and are being performed at an increasing rate <sup>1</sup>.

The overall incidence of complication after PLB rates from 1.2–6.8%, whereas post-biopsy bleeding represents the most serious complication and occurs in up to 10.9% of cas

es<sup>2,3</sup>. Arteriovenous fistula as a complication of PLB has been reported in 5.4%–52% of cases<sup>4,5</sup>. However, in recent medical literature data, the occurrence of arteriovenous fistulas or arterioportal fistulas following PLB has only been reported in liver transplant recipients<sup>6,7</sup>. The hepatic arteriovenous fistula, with its communication with skin on the site of skin puncture, has not been reported yet. We present a case with a delayed diagnosis of arteriovenous fistula as a complication of PLB manifested with chronic bleeding from the site of the puncture on the skin.

### Case report

A 35-year-old female patient was admitted to our institution with secondary anemia caused by chronic bleeding from a small skin wound in the right upper region of the abdomen. On admission, red blood cells (RBC) was  $2.4 \times 10^{12}/L$  [normal range (nr)  $3.8\text{--}5.8 \times 10^9/L$ ], hemoglobin of 59 g/L (nr 115–165 g/L), and hematocrit of 37% (nr 37–47%). All other laboratory parameters, including platelets, international normalized ratio (INR), partial thromboplastin time (PTT), liver enzymes, antinuclear antibodies, antineutrophil cytoplasmic antibodies, and parameters of inflammation, were in normal ranges.

It was reported in the patient's medical history that she underwent PLB with a fine needle 7 years ago due to slightly elevated liver enzymes. The histopathological finding suggested mild hepatitis with subsequent exclusions of hepatitis A, B, and C viral infections. After PLB, a granuloma, which occasionally bled, appeared at the puncture site on the skin. Due to the inability to heal, granuloma was treated with local excisions, antibiotics, and local skin transplantations in different healthcare institutions. Following these procedures, rib osteomyelitis has been diagnosed. After the failure of conservative treatment, the patient underwent resections of the right 11<sup>th</sup> rib and parts of the right rib arch in the region of the 7<sup>th</sup> and 8<sup>th</sup> ribs. The skin wound diminished over time but could not heal and close completely. Based on the suggestion of a hematologist and

dermatologist, who suspected the existence of an undefined autoimmune disease, the patient received treatment with cyclosporine and pulse doses of methylprednisolone with subsequent corticosteroid therapy. This kind of treatment failed, and the skin wound kept occasionally bleeding for years. Blood transfusions and blood product administration have been indicated periodically to correct the patient's anemic syndrome. The diagnostic procedures indicated by hematologists revealed hypogammaglobulinemia, factor XIII deficiency (46%), and severe CD4<sup>+</sup> T lymphocyte suppression. Coagulopathy, platelets dysfunctions disorders, and Ehlers-Danlos syndrome have been excluded. Meanwhile, a pathological vascularization in the right liver lobe was observed during one of the computed tomography (CT) scans, and embolization was attempted during selective angiography of the liver. One week before admission to our hospital, the patient experienced increased bleeding from the skin wound.

Upon patient admission to our hospital, blood transfusion, and administration of supportive therapy, a body CT scan revealed pathological communication of the right hepatic vein, portal branch for the anterior liver section, and posterior artery sectoral branch (Figure 1).

Throughout the skin wound, the fistulography was performed and confirmed the pathological vascularization in the right liver lobe. On this radiological examination, a pathological cavity diameter of  $40 \times 10$  mm was shown, which communicated with the arterial and venous system of the right liver lobe (Figure 2).

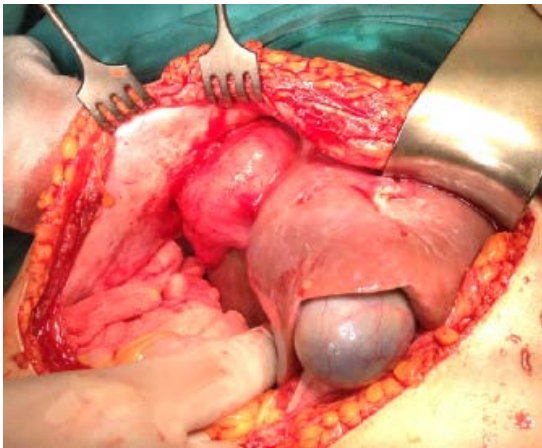
Due to a previously failed embolization and obvious late complication of PLB, it was concluded that the surgery would represent the safest treatment modality for the patient. During laparotomy, a tumor mass with a diameter of  $9 \times 6$  cm was found in the VI and VII liver segments (Figures 3 and 4). The communication between skin, liver, and tumor mass was evident. The tumor mass was removed with atypical resection of the VI and VII liver segments (Figure 5). Histopathological findings of the specimen showed granulation tissue with chronic inflammation and slight portal and



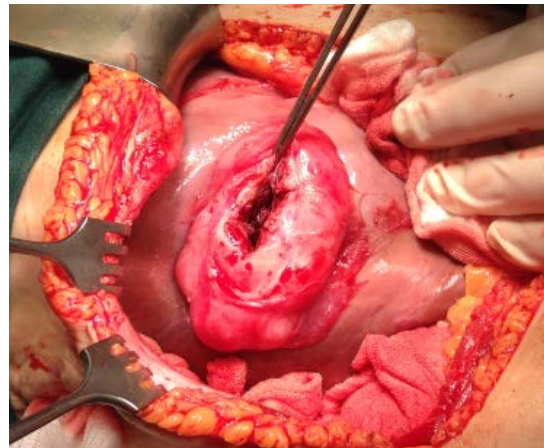
**Fig. 1 – Abdominal computed tomography scan with pathological communication between arterial, venous, and portal venous system of the right liver lobe.**



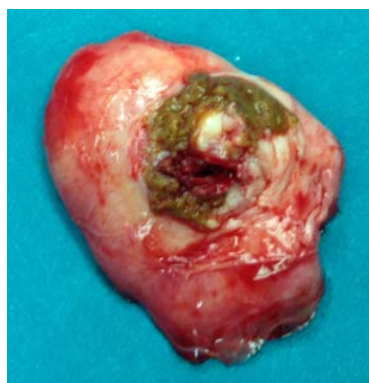
**Fig. 2 – The fistulography through the wound of the skin and pathological communication of the arterial and venous system of the right liver lobe.**



**Fig. 3 – Intraoperative finding of tumor formation connected with the right liver lobe.**



**Fig. 4 – Tumor formation with central cavitation and fistula's tract to the liver parenchyma.**



**Fig. 5 – Tumor specimen after excision and atypical liver resection.**

lobular inflammatory infiltrate. A reexploration was indicated on the second postoperative day due to acute hemorrhage. Bleeding was found at the cut parenchymal surface; afterward, liver segmentectomy was performed. The further postoperative

course was uneventful, and the patient was discharged from the hospital two weeks after the first operation. During follow-ups, one and three months after hospital discharge, there were no signs of bleeding, and laboratory findings were normal.

## Discussion

Complications after fine needle PLB are not frequent. The most frequent complication following PLB is postprocedural bleeding<sup>2,3</sup>. Hepatico-cutaneous fistula as a complication of PLB has not been described yet. However, such a complication has been reported after percutaneous radiofrequency ablation of hepatocellular carcinoma<sup>8</sup>. Pathological communication between liver arteries and/or portal system and hepatic veins (arterio-venous fistula) may occur due to the rupture of artery aneurism into the portal system, in primary or secondary liver malignancies, or liver trauma<sup>9</sup>. Iatrogenic arteriovenous liver fistulas have been reported after liver biopsies, percutaneous transhepatic cholangiography, and following transhepatic biliary drainage<sup>9-12</sup>. The first literature data on arteriovenous liver fistula as a complication of PLB was described in 1967<sup>11</sup>. After that report, several arteriovenous fistulas have been described, suggesting that the incidence of this kind of complication after liver biopsy is much higher than indicated by the scattered case reports<sup>13</sup>. Fortunately, these patients were asymptomatic, and shunts did not persist on the repeated angiography<sup>13</sup>. Other reports suggested that arteriovenous hepatic fistula, including arteriportal fistula, may be quiescent for a considerable time<sup>13,14</sup>. In addition, a large multicentre study evaluated complications of PLB and did not find any arteriovenous hepatic fistula<sup>12</sup>. However, there are several described cases in the recent literature of intrahepatic arteriportal fistula following PLB<sup>15-17</sup>. Our patient has been asymptomatic for a certain time, with unspecific clinical signs. Perhaps that was the reason for misdiagnosis and inadequate treatment, including the fact that the majority of arteriovenous hepatic fistula could not be seen on single angiography<sup>13,14</sup>. However, “over-treatment” in the presented patient in this perspective was unnecessary.

In proven hepatic arteriovenous (or arteriportal) fistula, the treatment modality includes embolization and surgical procedure<sup>18,19</sup>. The specificity of the presented patient was the presence of not only communication between the hepatic artery and portal system but the communication with the right hepatic vein. Moreover, the site of PLB on the skin and skin wound has been transformed in the fistula tract, which communicates with the liver and intrahepatic

arteriovenous fistula. Delayed diagnosis in our patient led to the “over-treatment” with multiple surgical procedures and chronic anemia. An accurate diagnosis of hepatico-cutaneous fistula in the presented patient was made with fistulography, one of the simplest diagnostic procedures. Fistulography should not be avoided in favor of imaging procedures such as magnetic resonance imaging and/or CT scan. However, a CT scan was necessary to confirm communication between the branch of the right hepatic artery, right portal, and hepatic vein. Bearing in mind the assumption that fistula embolization would be unsuccessful, our multidisciplinary team decided that surgical treatment was the only safe treatment with the goal of definitive care for our patients. Although parenchymal-sparing liver resections have shown comparable safety and efficacy compared with anatomic resections<sup>20,21</sup>, the patient experienced early postoperative bleeding after atypical liver resection.

Although the PLB is a safe and effective procedure, the rate of bleeding complications ranges between 0% and 25%, with the vast majority of studies reporting rates of bleeding under 2%<sup>3</sup>. If this complication occurs, the bleeding usually stops spontaneously, which was not the case with our patient. Furthermore, our patient had complicated intrahepatic fistula formation along with the communication of the skin site of the puncture. That is the first described case of unusual complication after PLB, and, as such, it is necessary to reconsider all possible consequences of PLB. In this term, with the adequate implementation of diagnostic procedures and timely treatment, it is possible to avoid any unnecessary and inappropriate approaches.

## Conclusion

Arteriovenous intrahepatic fistula is a rare complication of PLB and may be complicated by chronic bleeding and a formation of the fistula tract with the site of puncture on the skin. The presented patient is the first one described in the literature with this complication following PLB. Fistulography may be a useful diagnostic tool, and the initial treatment should be embolization of the intrahepatic fistula. In case of failure of embolization, the liver resection should be performed by adapted and individualized liver resection (anatomic or parenchyma sparing) to the site of the intrahepatic fistula.

## R E F E R E N C E S

1. Kose S, Ersan G, Tatar B, Adar P, Sengel BE. Evaluation of percutaneous liver biopsy complications in patients with chronic viral hepatitis. *Eurasian J Med* 2015; 47(3): 161–4.
2. Mueller M, Kratzer W, Oeztuerk S, Wilhelm M, Mason RA, Mao R, et al. Percutaneous ultrasonographically guided liver punctures: an analysis of 1961 patients over a period of ten years. *BMC Gastroenterol* 2012; 12:173.
3. Midia M, Odedra D, Shuster A, Midia R, Muir J. Predictors of bleeding complications following percutaneous image-guided liver biopsy: a scoping review. *Diagn Interv Radiol* 2019; 25(1): 71–80.
4. Okuda K, Musha H, Nakajima Y, Takayasu K, Suzuki Y, Morita M, et al. Frequency of intrahepatic arteriovenous fistula as a sequela to percutaneous needle puncture of the liver. *Gastroenterology* 1978; 74(6): 1204–7.
5. Hellekant C, Olint T. Vascular complications following needle puncture of the liver. An angiographic investigation in the rabbit. *Acta Radiol Diagn* 1973; 14(5): 577–82.
6. Jabbour N, Reyes J, Zajko A, Nour B, Tzakis AG, Starzl TE, et al. Arteriportal fistula following liver biopsy. Three cases occurring in liver transplant recipients. *Dig Dis Sci* 1995; 40(5): 1041–4.
7. Zhang DS, Metwalli Z, Hussain KB. Arterial-Portal Fistula After Percutaneous Liver Biopsy in Hepatic Allograft Causing Hemobilia and Pancreatitis. *Clin Gastroenterol Hepatol* 2017; 15(1): e3–e4.

8. *Huang HK, Lin CH, Hsu CC, Yu JC, Liu YC, et al.* Hepatocutaneous fistula: a complication after radiofrequency ablation therapy for hepatocellular carcinoma. *N Z Med J* 2007; 120(1255): U2562.
9. *Fagot H, Guieu M, Zemour J.* Intra-hepatic arterio-venous fistula after penetrating liver injury. *J Visc Surg* 2020; 157(5): 446–7.
10. *Tanaka H, Iwai A, Sugimoto H, Yoshioka T, Sugimoto T.* Intrahepatic arterioportal fistula after blunt hepatic trauma: case reports. *J Trauma* 1991; 31(1): 143–6.
11. *Preger L.* Hepatic arteriovenous fistula after percutaneous liver biopsy. *Am J Roentgenol Radium Ther Nucl Med* 1967; 101(3): 619–20.
12. *Piccinino F, Sagnelli E, Pasquale G, Giusti G.* Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J Hepatol* 1986; 2(2): 165–73.
13. *Hellekant C.* Vascular complications following needle puncture of the liver. Clinical angiography. *Acta Radiol Diagn (Stockh)* 1976; 17(2): 209–22.
14. *Wallace S, Medellin H, Nelson RS.* Angiographic changes due to needle biopsy of the liver. *Radiology* 1972; 105(1): 13–8.
15. *Bloom-Feshbach K, Fischman A, Leong J.* A Case of Intrahepatic Arterioportal Fistula After Liver Biopsy. *J Clin Gastroenterol* 2016; 50(3): 266–7.
16. *Invaki T, Miyatani H, Yoshida Y, Matsuura K, Suminaga Y.* Gastric variceal bleeding caused by an intrahepatic arterioportal fistula that formed after liver biopsy: a case report and review of the literature. *Clin J Gastroenterol* 2012; 5(2): 101–7.
17. *Kakati BR, Pedersen MR, Chen SY, Hirsch KS, Berggreen PJ, Seetharam AB.* Hepatic arterioportal fistula presenting as gastric variceal hemorrhage. *J Gastrointest Liver Dis* 2014; 23(2): 211–4.
18. *Isik FF, Greenfield AJ, Guben J, Birkett D, Menzoiian JO.* Iatrogenic arterioportal fistulae: diagnosis and management. *Ann Vasc Surg* 1989; 3(1): 52–5.
19. *Sniderman KW, Morse SS, Rapoport S, Ross GR.* Hemobilia following transhepatic biliary drainage: Occlusion of an hepatoportal fistula by balloon tamponade. *Radiology* 1985; 154:827.
20. *Bezmarevic M, Engelsman AF, Besselink MG.* Laparoscopic segment IVa liver resection: the diamond technique - a video vignette. *Colorectal Dis* 2019; 21(9): 1099–1100.
21. *Moris D, Ronnekleiv-Kelly S, Rahmema-Azar AA, Felekouras E, Dillhoff M, Schmidt C, et al.* Parenchymal-Sparing Versus Anatomic Liver Resection for Colorectal Liver Metastases: a Systematic Review. *J Gastrointest Surg* 2017; 21(6): 1076–85.

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# Basophilic peripheral nerve inclusions in a patient with L144F *SOD1* amyotrophic lateral sclerosis

## Bazofilne inkluzije u perifernom nervu kod bolesnika sa L144F *SOD1* amiotrofičnom lateralnom sklerozom

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### Abstract

**Introduction.** Histopathological findings of various inclusions were reported in the central nervous system of amyotrophic lateral sclerosis (ALS) patients but not in the peripheral nerves. **Case report.** We present a 66-year-old man with lower limb weakness, with later development of weakness in the upper limbs and loss of sphincter control. Neurological examination showed the affection of both upper and lower motor neurons. He had paresthesia on the left side of his body and socks-distribution numbness. Histopathology of the sural nerve and genetic report showed basophilic periodic acid-Schiff (PAS)-positive intra-axonal inclusions and heterozygous L144F mutation in the exon 5 of the *SOD1* gene. **Conclusion.** It seems that the presence of the basophilic peripheral nerve inclusions may suggest a diagnosis of *SOD1*-positive ALS.

### Key words:

amyotrophic lateral sclerosis; diagnosis; genes; histological techniques; mutation; staining and labeling; superoxide dismutase.

### Apstrakt

**Uvod.** Postoje izveštaji o histopatološkim nalazima različitih inkluzija u centralnom nervnom sistemu kod bolesnika sa amiotrofičnom lateralnom sklerozom (ALS), ali ne i u perifernim nervima. **Prikaz bolesnika.** Prikazan je bolesnik star 66 godina sa slabošću donjih ekstremiteta, kasnijim razvojem slabosti i gornjih ekstremiteta i gubitkom kontrole sfinktera. Neurološkim pregledom utvrđeni su znakovi oštećenja i gornjeg i donjeg motornog neurona. Pored parestezija leve strane tela, bolesnik je osećao i utrnulost sa distribucijom po tipu „čarapa”. Histopatološkom analizom suralnog nerva i genetskim analizama utvrđene su bazofilne *periodic acid-Schiff* (PAS)-pozitivne inkluzije unutar aksona i heterozigotna mutacija L144F u egzonu 5 *SOD1* gena. **Zaključak.** Prisustvo bazofilnih inkluzija u perifernom nervu može ukazati na dijagnozu *SOD1*-pozitivne ALS.

### Ključne reči:

amiotrofijska lateralna skleroza; dijagnoza; geni; histološke tehnike; mutacija; bojenje preparata; superoksid dismutaza.

### Introduction

Around 5–10% of patients with amyotrophic lateral sclerosis (ALS) have a genetic basis for the disease. Mutations in the *SOD1* gene are one of the most common causes of ALS, accounting for around 23% of familial ALS and about 7% of apparently sporadic ALS in all studies. Over 185 disease-associated variations in *SOD1* have been identified so far <sup>1</sup>.

From 5% to 10% of ALS individuals have at least one additional family member affected by ALS (so-called familial ALS – fALS), but the majority of ALS cases (90–95%) occur in people with no prior family history (so-called sporadic ALS – sALS) <sup>2</sup>. In both fALS and sALS, genetic causes of the disease may be established, which is marked as hereditary ALS. Various histopathological changes have been demonstrated in *SOD1* fALS patients, both in the central nervous system (CNS) and peripheral nervous system (PNS) <sup>2, 3</sup>. Spheroid shape and



eosinophilic inclusions containing neurofilaments in the anterior horn cells and Betz cells were observed in fALS patients with the I113T *SOD1* mutation<sup>4</sup>. There are numerous indicators of PNS involvement in fALS, especially in patients with the *SOD1* mutations<sup>2</sup>. Examinations of the sural nerve showed an axonal loss, *tomacula*-like myelin thickenings, and inflammatory infiltrate<sup>5</sup>. In a patient with the D90A *SOD1* mutation, a sural nerve biopsy showed loss of large myelinated fibers and degenerative changes of myelinated and unmyelinated fibers<sup>6</sup>.

To the best of our knowledge, we present a unique case of apparently sALS patient with the L144F mutation in the *SOD1* gene who had intra-axonal basophilic periodic acid-Schiff (PAS)-positive inclusions.

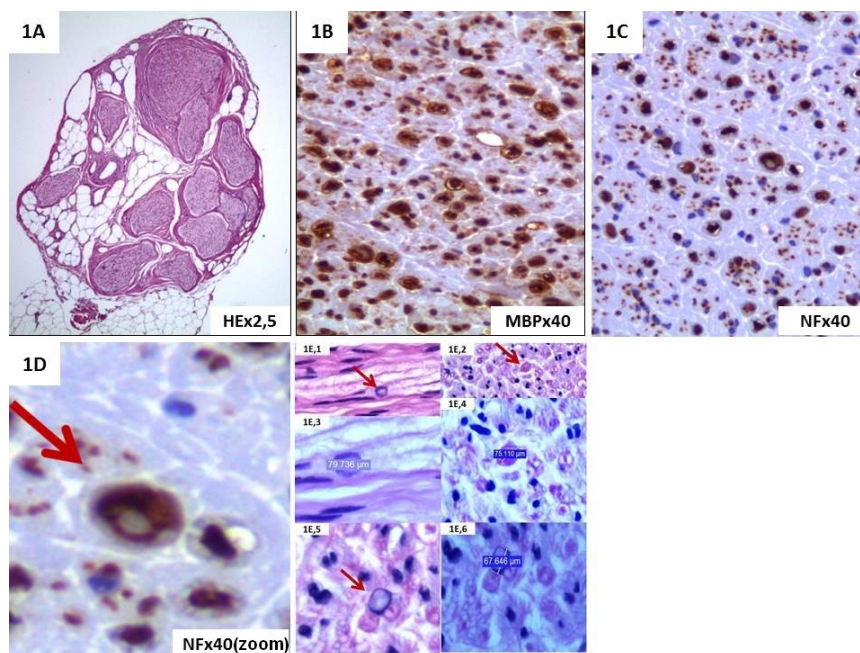
### Case report

A 66-year-old male presented with a five-year history of progressive weakness with atrophy of distal muscles, especially of both legs, as well as a slight weakness and distal muscle atrophy in both arms, rare fasciculations, and loss of sphincter control. On admission, he was not able to walk.

A neurological examination of the patient revealed bilateral distal muscle atrophy and rare fasciculations with generalized mild weakness in the upper limbs. In the lower limbs, the patient had bilateral distal muscle atrophy and provoked fasciculations with severe generalized weakness.

The tendon reflexes were brisk, except for ankle jerks which were diminished, and the presence of bilateral Babinski sign was noticed. Standing and walking were impossible. He had socks-distribution paresthesia. The urinary catheter was placed due to incontinence. The Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-r) was 38/48. The mini-mental status examination (MMSE) score was normal. Electromyography showed neurogenic changes with actual denervation activity at rest in the cervical and lumbosacral regions. A nerve conduction study showed axonal sensory and motor neuropathy, which was more pronounced in the lower limbs. Laboratory and cerebrospinal fluid (CSF) studies were normal. Magnetic resonance imaging of the brain and spinal cord were unremarkable. The conclusion was that the patient fulfilled El Escorial revised criteria for probable ALS, with atypical clinical features such as axonal neuropathy and bladder dysfunction. In the next three months, nerve and muscle biopsies and genetic examinations were performed. The patient died five months later at the age of 67 due to cardiopulmonary arrest.

A biopsy of the right gastrocnemius muscle revealed a neurogenic lesion, rare and atrophic end-stage muscle fibers, and a dominant presence of fatty and connective tissue. Sural nerve biopsy showed the following: fatty and connective tissue infiltration (Figure 1A); intense reduction of small and large myelinated fibers in number; secondary remyelination



**Fig. 1 – The sural nerve biopsy: A) Interfascicular infiltration with fat and connective tissue (HE,  $\times 2,5$ ); B) Intense reduction in the number of small and large myelin fibers, secondary remyelination and regeneration clusters (MBP,  $\times 40$ ); C) Axonal degeneration (NF,  $\times 40$ ); D) A cross-section of a fiber stained for the NF ( $\times 40$ -zoom) shows an empty space at the inclusion site (red arrow); E) (1–6): Oval inclusions of variable diameters, visible on transverse and longitudinal sections ( $\times 40$ ): 1E1) Basophilic inclusions (red arrow) on a longitudinal section (HE); 1E2) PAS-positive inclusions (red arrow); 1E3) PAS-positive inclusions of diameter 79  $\mu\text{m}$  (intra-axonally) on a longitudinal section; 1E4) PAS-positive inclusions of diameter 75  $\mu\text{m}$  (intra-axonally) on a cross-section; 1E5) The inclusion (red arrow) fills the space of the axon (PAS); 1E6) PAS-positive inclusions of diameter 67  $\mu\text{m}$  (intra-axonally) on a cross-section.**

HE – hematoxylin and eosin staining; MBP – myelin basic protein; NF – neurofilament; PAS – Periodic acid-Schiff.

and regeneration (Figure 1B), together with axonal degeneration (Figure 1C). Basophilic PAS-positive inclusions, 14–79 µm in diameter, were observed intra-axonally in several fascicles. These inclusions were PAS-positive (Figures 1D and 1E). The inclusions were described as polyglucosan body-like alterations. Genetic analysis showed a pathogenic heterozygous missense L144F mutation in the exon 5 of the *SOD1* gene, which led to the final diagnosis of genetic ALS.

## Discussion

We presented a patient with the L144F mutation of the *SOD1* gene and atypical presentation of ALS. Polyglucosan body-like inclusions were seen on the peripheral nerve biopsy. Polyglucosan bodies are pathologic hallmarks of a very rare neurometabolic disorder, i.e., adult polyglucosan body disease (APBD) with peripheral neuropathy, upper motor neuron signs, neurogenic bladder, and dementia. Many patients lack one or more of these features. The diagnosis is confirmed based on the activity of the branched enzyme and genetic analysis. White matter involvement in APBD may be extensive<sup>4</sup>. Very rare manifestations include ALS-like features registered in only six patients<sup>7</sup>. Clinical presentation of APBD and *SOD1* L144F ALS may be similar. Patients with L144F have a slowly progressive disease with lower limb onset, combined upper and lower motor neuron signs, and atypical clinical features, e.g., sphincter and sensory disturbances<sup>8</sup>.

APBD may be diagnosed incorrectly based only on the presence of polyglucosan bodies, which is a nonspecific finding<sup>7</sup>. We registered a reduction of small and large myelinated fibers in number, axonal degeneration, as well as remyelination and regeneration that have been previously described in ALS patients<sup>5,6</sup> with PAS-positive basophilic inclusions in

the sural nerve specimens that have not been described in *SOD1* patients so far. PAS-positive inclusions resembled findings in APBD, so only genetic analyses were able to distinguish between APBD and ALS<sup>9</sup>. Based on the clinical presentation, needle electromyography, and even nerve biopsy, it is not always possible to distinguish with certainty between APBD and L144F *SOD1* ALS, so it is necessary to perform genetic analyses that will confirm APBD or ALS.

It is well known that neuronal inclusions containing aggregated *SOD1* are the pathological hallmark of *SOD1* ALS seen both in patients and in transgenic animal models overexpressing mutant *SOD1* protein. Some *SOD1* ALS patients, including those with L144F mutation, have larger skein-like and Lewy body-like inclusions, in addition to smaller misfolding *SOD1* inclusions<sup>10</sup>. We were not able to perform additional enzymatic and genetic analyses for adult polyglucosan body disease, as well as immunohistochemical studies covering epitopes across the entire *SOD1* protein to see if the inclusions seen in our patients contain *SOD1* aggregates.

## Conclusion

Basophilic peripheral nerve inclusions may be associated with *SOD1*-positive ALS.

## Conflict of interest

The authors report no conflict of interest.

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## REFERENCES

1. Mejszini R, Flynn LL, Pitout IL, Fletcher S, Wilton SD, Akkari PA. ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? *Front Neurosci* 2019; 13: 1310.
2. Gentile F, Scarlino S, Falzone YM, Lunetta C, Tremolizzo L, Quattrini A, et al. The Peripheral Nervous System in Amyotrophic Lateral Sclerosis: Opportunities for Translational Research. *Front Neurosci* 2019; 13: 601.
3. Suzuki M, Yasui K, Ishikawai H, Nomura M, Watanabe T, Mikami H, et al. Familial amyotrophic lateral sclerosis with Cys111Tyr mutation in Cu/Zn superoxide dismutase showing widespread Lewy body-like hyaline inclusions. *J Neurol Sci* 2011; 300(1–2): 182–4.
4. Berkehoff M, Weis J, Schroth G, Sturzenegger M. Extensive white-matter changes in case of adult polyglucosan body disease. *Neuroradiology* 2001; 43(3): 234–6.
5. Luigietti M, Conte A, Del Grande A, Bisogni G, Romano A, Sabatelli M. Sural nerve pathology in ALS patients: a single-centre experience. *Neurol Sci* 2012; 33(5): 1095–9.
6. Giannini F, Battistini S, Mancuso M, Giuseppe G, Ricci C, Volpi N, et al. D90A-SOD1 mutation in ALS: The first report of heterozygous Italian patients and unusual findings. *Amyotroph Lateral Scler* 2010; 11(1–2): 216–9.
7. Milde P, Guccion JG, Kelly J, Locatelli E, Jones RV. Adult polyglucosan body disease. *Arch Pathol Lab Med*. 2001; 125(4): 519–22.
8. Marjanović IV, Selak-Djokić B, Perić S, Janković M, Arsenijević V, Basta I, et al. Comparison of the clinical and cognitive features of genetically positive ALS patients from the largest tertiary center in Serbia. *J Neurol* 2017; 264(6): 1091–8.
9. Mochel F, Schiffmann R, Steenweg ME, Akman HO, Wallace M, Sedel F, et al. Adult polyglucosan body disease: Natural History and Key Magnetic Resonance Imaging Findings. *Ann Neurol* 2012; 72(3): 433–41.
10. Forsberg K, Graffmo K, Pakkenberg B, Weber M, Nielsen M, Marklund S, et al. Misfolded SOD1 inclusions in patients with mutations in *C9orf72* and other ALS/FTD-associated genes. *J Neurol Neurosurg Psychiatry* 2019; 90(8): 861–9.

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## Mucous membrane pemphigoid – a report of four cases

### Pemfigoid mukozne membrane

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#### Abstract

**Introduction.** Mucous membrane pemphigoid (MMP) is a rare autoimmune, chronic inflammatory disease that affects mucous membranes, most commonly the eyes and mouth, with or without skin involvement. It is a complex disease with several complications, including scarring, especially on conjunctival mucosa, that can lead to visual loss. **Case report.** We report four patients (two men and two women) with MMP. In all patients, the disease started between seventy and eighty years of age. The diagnosis was confirmed based on clinical appearance, histology, direct and indirect immunofluorescence studies, indirect split skin technique, and enzyme-linked immunosorbent assay (ELISA) test. The majority of lesions were on the gums and buccal mucosa; one patient had laryngeal involvement and a lesion on the umbilicus. No ocular involvement and no malignancy were detected. Direct immunofluorescence tests revealed continuous linear IgG deposition in the basal membrane zone in two patients, and they were treated with oral nicotineamide and tetracycline hydrochloride. In two patients, we detected IgG along with IgA linear deposition; they received treatment with methylprednisolone. Complete remission was achieved in all patients. **Conclusion.** Early diagnosis and an adequate therapeutic approach are necessary for the MMP treatment in long-term disease control and reduction of disease-related complications.

#### Key words:

diagnosis; bullous pemphigoid; enzyme-linked immunosorbent assay; fluorescent antibody technique, direct; remission induction.

#### Apstrakt

**Uvod.** Pemfigoid mukozne membrane (PMM) je retka autoimunska, hronična inflamacijska bolest, koja utiče na sluzokožu, najčešće očiju i usta, sa ili bez promena na koži. To je složena bolest sa nekoliko komplikacija, koje uključuju ožiljke, posebno na sluzokoži konjunktiva, što može dovesti do gubitka vida. **Prikaz bolesnika.** Prikazana su četiri bolesnika (dva muškarca i dve žene) sa PMM. Kod svih bolesnika bolest se javila u osmoj deceniji života. Dijagnoza je potvrđena na osnovu kliničke slike, histološkog nalaza, analiza direktno i indirektno imuno fluorescencije, indirektno *split-skin* tehnike i testa – *enzyme-linked immunosorbent assay* (ELISA). Većina lezija nalazila se na desnama i sluzokoži obraza, a kod jednog bolesnika bio je zahvaćen grkljan i postojala je lezija na pupku. Oči nisu bile zahvaćene i nisu primećene promene sumnjive na malignitet. Kod dva bolesnika testovima direktno imuno fluorescencije otkriveno je kontinuirano linearno taloženje IgG u zoni bazalne membrane i oni su lečeni oralnim nikotianimidima i tetraciklin hidrohloridom. Kod dva bolesnika otkrili smo linearno nataložene IgG zajedno sa IgA i oni su lečeni metilprednizolonom. Kod svih bolesnika je ostvarena potpuna remisija. **Zaključak.** Za dugoročnu kontrolu bolesti i smanjenje komplikacija povezanih sa PMM neophodni su rana dijagnoza i multidisciplinarni pristup u lečenju obolelih.

#### Ključne reči:

dijagnoza; pemfigoid, bulozni; elisa; fluorescentna antitela, direktna tehnika; remisija, indukcija.

#### Introduction

Mucous membrane pemphigoid (MMP) is a rare autoimmune, chronic inflammatory disease that affects mucous

membranes, most commonly the oral and conjunctival, but also nasal, oropharyngeal, laryngeal, esophageal, and anogenital, with or without skin involvement<sup>1-3</sup>. MMP lesions are prone to infection, causing fibrosis and granulation tissue

formation. They often heal with scars which can cause permanent disfigurement and complications such as dysphagia and blindness<sup>1,4</sup>. That is the reason why synonyms for MMP in the literature include benign MMP but also cicatricial (scarring) pemphigoid and ocular cicatricial pemphigoid. The incidence is 1–5 cases per million individuals<sup>1</sup>. Elderly females are commonly affected. The mean age of onset is between 50 and 80 years<sup>5</sup>. Changes in the oral mucosa present as erythematous patches, blisters, erosions, and ulcerations, located most commonly on gingival and palatal mucosa. The diagnostic process involves a combination of clinical, histological, and immunopathological studies<sup>6</sup>. Dentists and ophthalmologists can be the first to suspect or diagnose this rare disease because of mucosal involvement<sup>7,8</sup>. Direct immunofluorescence (DIF) tests of mucous membranes detect continuous linear deposition of immunoglobulins (Ig) G and/or IgA and/or complement (C3) along the basement membrane zone (BMZ)<sup>6</sup>. To detect circulating autoantibodies to the BMZ, indirect immunofluorescence (IIF) tests with normal human skin as the substrate are usually performed; however, autoantibodies are detected in only 17–53% of MMP cases<sup>6</sup>. The IIF technique can be used to cause separation of the BMZ at the level of *lamina lucida* with 1M NaCl because it might be helpful to differentiate autoantigens located on the epidermal side of the BMZ from those located on the dermal side<sup>3,6</sup>. Enzyme-linked immunosorbent assays (ELISAs) are also widely used to detect autoantibodies directing specific

autoantigens – bullous pemphigoid antigens BP230 (BPAg1) and BP180 (BPAg2)<sup>3</sup>.

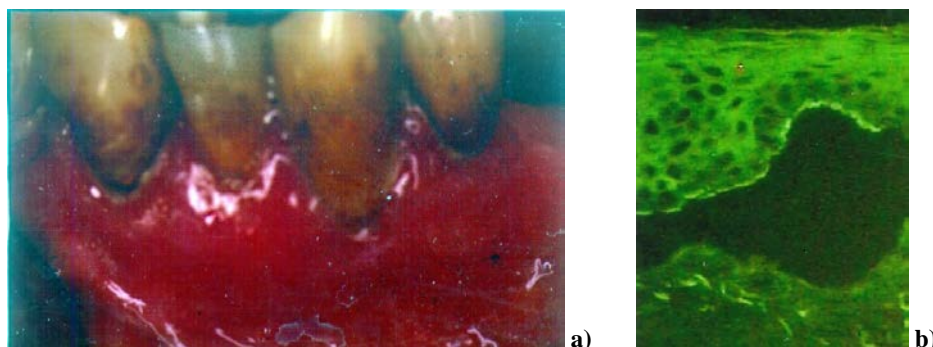
## Methods

### Case 1

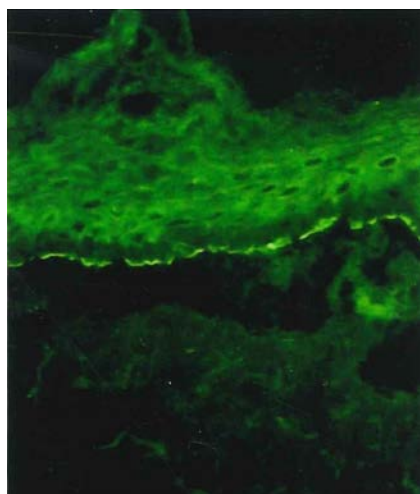
A 75-year-old man presented with a lower gingival painful ulceration that appeared 4 months earlier (Figure 1a). The DIF test revealed continuous linear IgG and IgA depositions in the BMZ. On the IIF split skin (ss) test (ssIIF), there was IgA binding to the roof (epidermal side) of the separated skin (Figure 1b). IIF showed circulating IgG anti-BMZ antibodies detected to a titer of 1 : 10. ELISA test showed positive BP180. The patient was treated with methylprednisolone 0.5 mg/kg, nicotinamide [1 g/daily(d)], and tetracycline hydrochloride (1 g/d) in combination with topical triamcinolone.

### Case 2

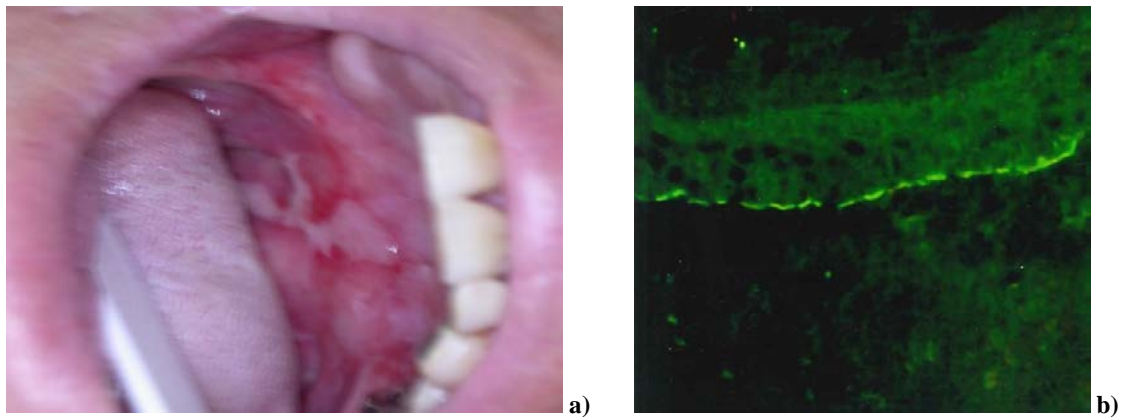
A 78-year-old man presented with a one-year history of a lesion on the gingival mucosa. The DIF test revealed a continuous linear IgG and C3, i.e., BMZ. On the ssIIF test, there was IgG binding to the roof (epidermal side) of the separated skin (Figure 2). IIF showed circulating IgG anti-BMZ antibodies detected to a titer of 1 : 80. ELISA test showed positive BP180. The patient was treated with nicotinamide (1 g/d) and tetracycline hydrochloride (1 g/d) in combination with topical triamcinolone.



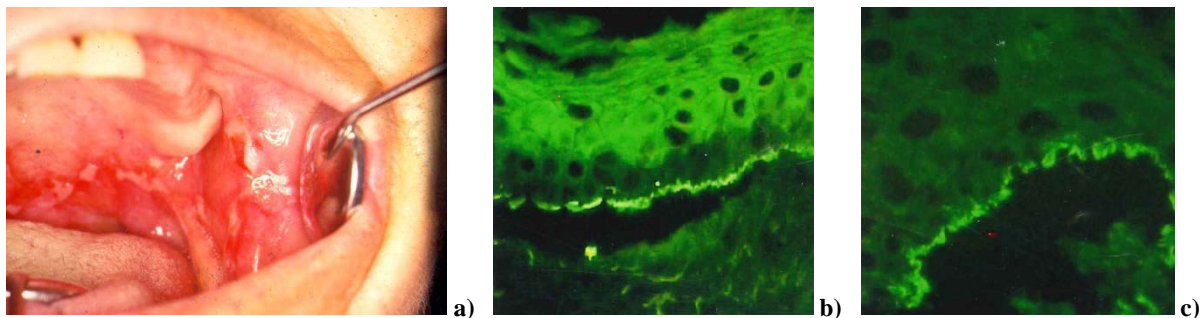
**Fig. 1 – a) Ulceration of the lower gingival mucosae; b) Indirect split skin: IgA – Linear basement membrane zone (Roof).**



**Fig. 2 – Indirect split skin: IgG – Linear basement membrane zone (Roof).**



**Fig. 3 – a) Multiple erosions of the buccal mucosae and sublingual; b) Indirect split skin: IgG –Linear basement membrane zone (Roof).**



**Fig. 4 – a) Diffuse irregular erosions and ulceration with pseudomembranes on the buccal mucosae and hard palate; b) and c) Indirect split skin: IgG – Linear basement membrane zone (BMZ) (Roof) and IgA – Linear BMZ (Roof).**

#### Case 3

A 71-year-old woman presented with a one-year history of lesions on the epiglottis, buccal and palatal mucosa (Figure 3a). The DIF test detected continuous linear IgG in the BZM. IIF testing of the patient's serum showed circulating IgG anti-BMZ antibodies detected to a titer of 1 : 100. The ssIIF test demonstrated that there was IgG binding to the roof of the separated skin (Figure 3b). ELISA test showed positive BP180. The patient was treated with methylprednisolone 0.5 mg/kg and topical triamcinolone.

#### Case 4

A 70-year-old woman presented with a three-month history of lesions on the gingival and buccal mucosa (Figure 4a), as well as on the umbilicus. The DIF revealed continuous linear IgG deposition and discontinuous IgA and C3 depositions in the BMZ. The ssIIF test demonstrated that the immunoglobulins were bound to the epidermal side of the separated skin (Figures 4b and 4c). IIF detected circulating IgG (titer 1 : 200) and IgA (titer 1 : 10) anti-BMZ antibodies. ELISA test was negative. She was treated with methylprednisolone (40 mg/d) and topical triamcinolone.

#### Discussion

The first case of MMP was reported by Wickmanns<sup>9</sup> in 1794 in a female patient. MMP is more commonly observed in women than men, with a male-to-female ratio of nearly

1 : 2<sup>1,4</sup>. The elderly population is most commonly affected by the disease; the majority of patients were in the age range of 60 to 80 years<sup>5</sup>.

In all our patients, the disease occurred with equal frequency among the sexes, but the sample size is too small to draw a conclusion. All patients had between seventy and eighty years, which mainly correlates with those described in the literature<sup>5</sup>. Cutaneous lesions on the head and upper trunk can be seen in a quarter of patients with MMP<sup>1</sup>. The oral and conjunctival mucosae are most frequently affected in MMP, followed by involvement of nasal, oropharyngeal, laryngeal, esophageal, and anogenital mucosa<sup>1</sup>. The gingiva is generally one of the oral sites with the greatest incidence after the palate and the buccal mucosa<sup>10</sup>. In the study of Arduino et al.<sup>10</sup>, almost 83% of patients suffered from gingival lesions (both upper and lower), followed by the palate (34%), buccal mucosa (25.3%), alveolar ridge (11%), tongue (7.7%), and labial mucosa (7.7%). The eyes are affected in about 70% of all MMP cases<sup>8</sup>.

Interestingly, in our patients, there was an exclusive oral presentation with no evidence of other mucosal involvement, which is similar to the case reports of Di Zenzo et al.<sup>11</sup>. Only one patient had a lesion on the skin in the region of the umbilicus.

In the study of La Placa et al.<sup>5</sup>, which included 22 patients affected by MMP, 18% of the patients were found to have, as remarked, an associated cancer. We performed all

necessary clinical and laboratory tests, but no evidence of malignancies was found.

The First International Consensus on MMP recommended dividing patients into “low-risk” and “high-risk” groups based upon the site(s) of involvement, with “low-risk” patients defined as having only oral mucosal or oral and skin involvement<sup>12</sup>. “High-risk” patients were defined as having the involvement of the ocular, genital, nasopharyngeal, esophageal, and/or laryngeal mucosae, and they required more aggressive treatment<sup>13</sup>. According to this view, our patients belong to the “low-risk” group.

The exact etiology of MMP is unknown. However, there are a few reports of MMP triggered by medications (methyl dopa, clonidine, indomethacin, and D-penicillamine), viruses, ultraviolet light, and genetic predisposition such as HLA DQB1\*0301<sup>14</sup>.

Bullous pemphigoid antigen (BPAG) 1, 230 kd, BPAG2, 180 kd, laminin 5, laminin 6,  $\alpha_6$ -integrin subunit,  $\beta_4$ -integrin subunit, collagen VII, and also other basement membrane zone components such as proteins of unknown origin and function have been identified as MMP autoantibody targets<sup>13, 15</sup>. Egan et al.<sup>16</sup> demonstrated a positive association between the presence of anti-laminin 5 antibodies and underlying solid neoplasm in 29% of cases. DIF investigations showed characteristic continuous linear deposits of IgG, C3, and IgA in the epithelial BMZ<sup>5</sup>. Arduino et al.<sup>10</sup> found linear deposits of IgG in 93.4%, C3 in 82.8%, and IgA in 12.9% of cases.

IIF test performed on 1 M salt-split normal human skin substrate provides the highest sensitivity for autoantibodies in pemphigoid diseases<sup>17, 18</sup>. The epidermal (“roof”) or dermal (“floor”) sides of the artificial blister are the places where the antibodies bind<sup>18</sup>. “Roof”-binding antibodies target BP180 and BP230 are observed in BP, linear IgA-disease, pemphigoid gestationis, and anti-BP180-type MMP. Sensitivities for BP range between 73 and 84%<sup>18</sup>. All of our four patients had IgG depositions, and one of them had IgA deposition to the epidermal side of the artificial blister, and 3 out of 4 patients were BP180 positive.

The main criteria in choosing a therapy depend on the patient’s risk status, high or low. It is recommended that those with “low-risk” MMP (involvement of oral mucosae and skin) be initially treated by topical corticosteroids, tacrolimus, or cyclosporine, with the addition of systemic treatment, which includes tetracycline hydrochloride along with niacinamide, if needed<sup>12, 17</sup>. Those with “high-risk” MMP (involvement of the

eyes, esophagus, larynx, and urogenital region) should be treated with systemic corticosteroids as the first choice, in combination with immunosuppressive and/or anti-inflammatory agents such as dapson, azathioprine, cyclophosphamide or mycophenolate mofetil<sup>12, 15</sup>. Treatment strategies also vary according to several factors, such as the age of the patient, severity of the lesions, and the involved sites<sup>7</sup>.

As third-line therapy, according to the European guideline for the management and treatment of bullous pemphigoid, rituximab was recommended if conventional immunosuppressive drugs were ineffective, contraindicated, or showed unacceptable side effects<sup>19</sup>. There is also a small study in which three patients with oral involvement MMP were successfully treated with subcutaneous injections of etanercept 25 mg twice a week<sup>11</sup>.

However, most of the reported cases consisted only of small patient numbers, and the true benefit of such treatments is, therefore, not yet clear<sup>20</sup>.

It is important to note that good oral hygiene is a very important addition to standard therapy, and patients should be constantly motivated to carry out standard hygiene procedures.

Based on the above, the clinical presentation, and the response to therapy, we started the treatment of the first patient with oral nicotinamide and tetracycline hydrochloride, then we added methylprednisolone. The second patient was treated with oral nicotinamide and tetracycline hydrochloride. In all patients, topical corticosteroids were added to the therapy. The third and fourth patients were treated with systemic and topical corticosteroid therapy from the beginning. Complete remission was achieved in all patients.

## Conclusion

We have reported on four patients with an oral manifestation of MMP who have shown an uncommon clinical presentation of this rare disease. All patients are unique for having only the oral mucosa affected by the lesions, without the involvement of other mucous membranes. Only one patient had a few lesions on the skin. MMP should be suspected when changes in the oral cavity occur in people, especially those over 60 years of age. Early diagnosis and an adequate therapeutic approach are necessary for the MMP treatment in long-term disease control and reduction of disease-related complications.

## REFERENCES

1. Madgar O, Baniel A, Yarom N, Glikson E, Zeeli T, Sprecher E, et al. Mucous membrane pemphigoid–otorhinolaryngological manifestations: a retrospective cohort study. *Eur Arch Otorhinolaryngol*, 2020; 277(3): 939–45.
2. Ormond M, McParland H, Thakrar P, Donaldson ANA, Andiappan M, Cook RJ, et al. Validation of an Oral Disease Severity Score (ODSS) tool for use in oral mucous membrane pemphigoid. *Br J Dermatol* 2020; 183(1): 78–85.
3. Kamaguchi M, Iwata H. The Diagnosis and Blistering Mechanisms of Mucous Membrane Pemphigoid. *Front Immunol* 2019; 10: 34.
4. Lee J, Seiffert-Sinha K, Attwood K, Sinha AA. A Retrospective Study of Patient-Reported Data of Bullous Pemphigoid and Mucous Membrane Pemphigoid From a US-Based Registry. *Front Immunol* 2019; 10: 2219.
5. La Placa M, Balestri R, Tartari F, Sechi A, Ferrara F, Loi C, et al. Mucous Membrane Pemphigoid-Associated Malignancies: Case Series and a Brief Overview of the Literature. *Dermatol Pract Concept* 2019; 9(2): 119–25.
6. Mustafa MB, Porter SR, Smoller BR, Sitaru C. Oral mucosal manifestations of autoimmune skin diseases. *Autoimmun Rev* 2015; 14(10): 930–51.

7. *Shruthi R, Shruthi H, Vidya A, Subbas B, Sajad AB.* Pemphigus vulgaris presenting as bleeding gums: A case report. *J Turgut Ozal Med Cent* 2017; 24(3): 324–7.
8. *Georgoudis P, Sabatino F, Szentmary N, Palioura S, Fodor E, Hama-da S,* et al. Ocular Mucous Membrane Pemphigoid: Current State of Pathophysiology, Diagnostics and Treatment. *Ophthalmol Ther* 2019; 8(1): 5–17.
9. *Wickmanns JE.* Ideas on the diagnosis. Hanover: Helwig; 1894. p. 89.
10. *Arduino PG, Broccoletti R, Carbone M, Conrotto D, Pettigiani E, Giacometti S,* et al. Describing the gingival involvement in a sample of 182 Italian predominantly oral mucous membrane pemphigoid patients: A retrospective series. *Med Oral Patol Oral Cir Bucal* 2017; 22(2): e149–52.
11. *Di Zenzo G, Carrozzzo M, Chan LS.* Urban legend series: mucous membrane pemphigoid. *Oral Dis* 2014; 20(1): 35–54.
12. *Chan LS, Ahmed AR, Anhalt GJ, Bernauer W, Cooper KD, Elder MJ,* et al. The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic indicators. *Arch Dermatol* 2002; 138(3): 370–9.
13. *Neff AG, Turner M, Mutasim DF.* Treatment strategies in mucous membrane pemphigoid. *Ther Clin Risk Manag* 2008; 4(3): 617–26.
14. *Hasan S.* Desquamative gingivitis - A clinical sign in mucous membrane pemphigoid: Report of a case and review of literature. *J Pharm Bioallied Sci* 2014; 6(2): 122–6.
15. *Santi CG, Gripp AC, Roselino AM, Mello DS, Gordilbo JO, Marsillac PF,* et al. Consensus on the treatment of autoimmune bullous dermatoses: bullous pemphigoid, mucous membrane pemphigoid and epidermolysis bullosa acquisita - Brazilian Society of Dermatology. *An Bras Dermatol* 2019; 94(2 Suppl 1): 33–47.
16. *Egan CA, Lazarova Z, Darling TN, Yee C, Yancey KB.* Anti-epiligrin cicatricial pemphigoid: clinical findings, immunopathogenesis, and significant associations. *Medicine (Baltimore)* 2003; 82(3): 177–86.
17. *Ujii H, Iwata H, Yamagami J, Nakama T, Aoyama Y, Ikeda S,* et al. Committee for Guidelines for the Management of Pemphigoid Diseases (Including Epidermolysis Bullosa Acquisita). Japanese guidelines for the management of pemphigoid (including epidermolysis bullosa acquisita). *J Dermatol* 2019; 46(12): 1102–35.
18. *Witte M, Zillikens D, Schmidt E.* Diagnosis of Autoimmune Blistering Diseases. *Front Med (Lausanne)* 2018; 5: 296.
19. *Feliciani C, Joly P, Jonkman MF, Zambruno G, Zillikens D, Ioannides D,* et al. Management of bullous pemphigoid: the European Dermatology Forum consensus in collaboration with the European Academy of Dermatology and Venereology. *Br J Dermatol* 2015; 172(4): 867–77.
20. *Sacher C, Hunzelmann N.* Cicatricial pemphigoid (mucous membrane pemphigoid): current and emerging therapeutic approaches. *Am J Clin Dermatol* 2005

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# Application of polyether ether ketone in obturator telescopic prosthesis fabrication: a case report

## Primena polieter-eter-ketona u izradi opturator teleskop proteza

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### Abstract

**Introduction.** The retention, stability, and plaque resistance of the obturator prosthesis significantly impact the improvement of the patient's quality of life. The aim of this paper was to draw attention to the benefits of telescopic obturator prostheses made of polyether ether ketone (PEEK). **Case report.** We present an approach to the telescopic obturator fabrication using PEEK as the material of choice for the primary and secondary telescopic crowns, which are an integral part of the framework, in a 65-year-old patient with partial maxillectomy. The positioning of the teeth was made with a silicone key, and the featuring of the gingival part of the prosthesis was performed by a composite. **Conclusion.** PEEK is a polymer with good mechanical and chemical properties besides being biocompatible. An obturator prosthesis made of this material does not incur enormous fabrication costs, and its satisfactory functionality contributes to the improvement of patients' lives.

**Key words:** crowns; dental prosthesis; polyetheretherketone; polymers.

### Apstrakt

**Uvod.** Retencija, stabilnost i neprijemčivost za plak opturator proteza ima značajan uticaj na poboljšanje kvaliteta života pacijenata. Cilj rada bio je da se ukaže na prednosti teleskop opturator proteza izrađenih od polieter-eter-ketona (PEEK). **Prikaz bolesnika.** Prikazujemo način izrade teleskop opturator proteze od PEEK-a, kao izabranog materijala, kako za primarne tako i za sekundarne teleskop krune, koje su sastavni deo skeleta proteze, kod 65-godišnjeg muškarca sa parcijalnom maksilektomijom. Postava zuba urađena je pomoću silikonskog ključa, a ostatak proteze izveden je kompozitom. **Zaključak.** Pored biokompatibilnosti, PEEK je polimer koga odlikuju dobra mehanička i hemijska svojstva. Opturator proteze izrađene od tog materijala ne poskupljuju u velikoj meri proces izrade, a njihova zadovoljavajuća funkcionalnost doprinosi poboljšanju kvaliteta života pacijenata.

**Ključne reči:** krune; zubna proteza; polieter-eter-ketoni; polimeri.

### Introduction

Surgical treatments of oral cancers often result in changed anatomy of the oral cavity structures, thus changing the patient's normal functioning. Postoperative radiation therapy additionally impedes the functioning of the orofacial system due to a decreased salivary secretion rate; difficulties in taking food, swallowing, and speech impediments may also occur. Under such changed conditions in the oral cavity, the fabrication of the final prosthetic dental rehabilitation is more challenging. Due to maxillary defects, the retention and the stability of the obturator prosthesis (OP) are affected, and

postoperative radiation therapy additionally reduces the load-bearing ability of natural and reconstructed tissues<sup>1</sup>.

Regardless of the increasing number of publications in which the benefits of surgical reconstruction are discussed, the fabrication of OP to repair maxillary defects is still a widely applied method<sup>2</sup>. The advantages of this method are the following: a quick closure of the defect and dental rehabilitation, restoration of the normal functioning of the orofacial system, and easy monitoring of wound healing and recurrence<sup>3</sup>. Retention may be achieved by the remaining teeth or by osseointegrated implants in an edentulous jaw. Weaknesses of the OP are as follows: inadequate leaning of the pros



thesis against the adjacent structures, insufficient retention, and stability due to the missing load-bearing tissue<sup>4</sup>.

The surgical obturator may be fabricated postoperatively before radiation therapy, so the patient may speak, swallow, and/or eat normally. The necessary period for making the final OP is six months after radiation therapy so that the tissues may join and stabilize after the maxillectomy and radiotherapy<sup>5</sup>. The stability of the OP size is affected by the defect and the position of the remaining teeth. Besides the retention of the prosthesis on the remaining teeth, the base of the prosthesis must be extended as much as possible to the remaining structures in the oral cavity. The maximum extension of the OP ensures the transfer of chewing forces to the remaining part of the palatal and the alveolar ridge, thus reducing pressure on the adjacent tissue<sup>6</sup>. One of the main problems of OP is retention and stability. Telescope prostheses ensure good retention, and another positive characteristic of this material is that telescope crowns could be made from it, in combination with various conventional materials or with the same material<sup>7</sup>. Another equally important problem of acrylic OP is the accumulation of bacteria on the surface, causing infections. The nonadhesive surface made of this material reduces the binding potential of cells and proteins on it, thus indirectly reducing the accumulation of the bacteria and the potential for an infection to occur<sup>8</sup>.

### Case report

A male patient aged 65 had a partial maxillectomy on the left side due to maxillary carcinoma. Six months after the radiation therapy, the postoperative defect of the maxilla was detected at a clinical examination in the premolar and molar re-

gions on the left. The maxillary defect, classified as Class II by the Armani classification system, reached the medium section of the palate (Figure 1). The treatment plan was to fabricate a partial OP due to the presence of the teeth at the front, on both sides, left and right. The retention was secured by the telescope crowns on the first molar on the right side – 16, and on the canine tooth on the left side – 23. For the fabrication of the partial OP, a material based on polyether ether ketone (PEEK) (Biological High-Performance Polymer BioHPP, Bredent GmbH & Co.KG) was used. Primary and secondary telescope crowns were made of PEEK, including the framework of the prosthesis. At Stage I, the impression was made by a standard tray and alginate, with the prior protection of the obturator section by Vaseline™ gauze for making the individual tray. After grinding teeth 16 and 23, impressions were done by adding silicone for the primary telescope crown creation. Then, temporary acrylic crowns were made and cemented, and the surgical obturator was returned. At Stage II, primary telescope crowns were placed on teeth 16 and 23; the impression was taken by the individual tray with the light body addition silicone, with the prior protection of the obturator section with Vaseline™ gauze (Figure 2). At Stage III, jaw relationships with the primary telescope crowns were determined within wax patterns (Figure 3). At Stage IV, the trial positioning of the teeth was performed. After the trial positioning of the teeth, a silicone key was made in the laboratory (Figure 4), and after that, the framework of the prosthesis started, forming secondary telescope crowns. After pressing the framework made of BioHPP, composite facets were placed in accordance with the previously created silicone key (Figure 5). Then, the featuring of the gingival portion and the polishing of the very prosthesis were performed (Figure 6). At Stage V, the delivery



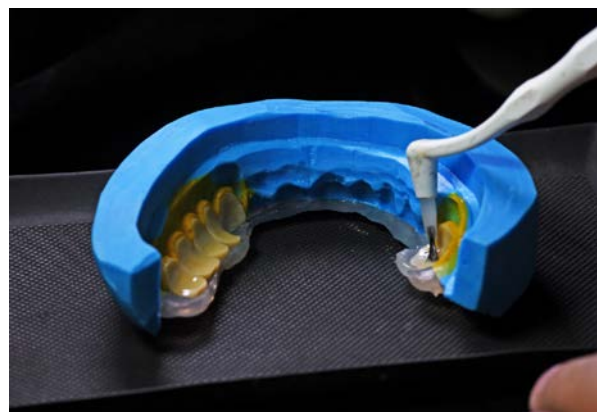
**Fig. 1 – The maxillary defect.**



**Fig. 2 – Individual tray.**



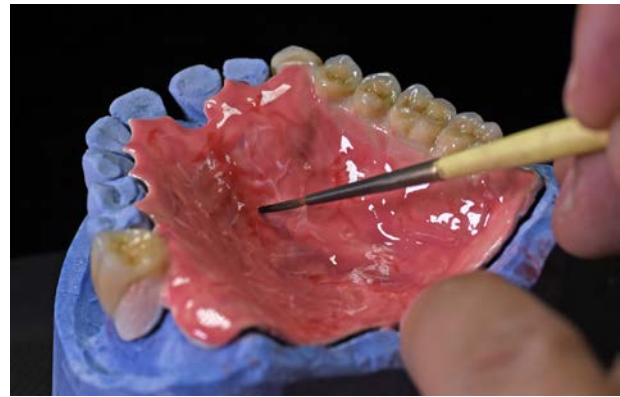
**Fig. 3 – Wax patterns with primary telescope crowns.**



**Fig. 4 – Silicone key.**



**Fig. 5 – Positioning of the composite facets according to the silicone key.**



**Fig. 6 – Gum and teeth characterization.**



**Fig. 7 – Telescope obturator prosthesis.**



**Fig. 8 – Telescope obturator prosthesis weight.**

of the telescopic prosthesis, identical to the delivery of the conventionally combined prosthetic dental rehabilitation, was performed (Figure 7). Before cementing the primary telescope crowns, it was necessary to protect the space between the primary and the secondary telescope crowns from unwanted penetration of definitive cement for primary crowns fixation. The precise obturation of the space between these two crowns may be ensured by wax or light body addition silicones. Dual composite cement (Multilink Automix, Ivoclar Vivadent) was used to cement the primary telescope crowns. After cementing, the surplus cement was removed, and the delivery of the OP was performed. The decreased weight of the telescope OP was noticed (Figure 8).

### Discussion

The fabrication of the OP has still been widely applied in repairing maxillary defects after maxillectomy. Besides the aesthetic aspect, the restoration of the normal functioning of the orofacial system is necessary for patients' quality of life. The purpose of the OP is to repair defects that have occurred due to maxillectomy<sup>9</sup>.

Advantages are reflected in the following: simple repair of the defect and dental rehabilitation, restoration of the oropharyngeal function, and easier monitoring of both the epithelialization of the wound inflicted by the surgery and the occurrence of a recidivation<sup>10</sup>. Patients often experience dif-

ficulties with chewing, swallowing, nasal speech, and overall appearance, all resulting in the patients' dissatisfaction<sup>11</sup>.

Depprich et al.<sup>6</sup> have mentioned acrylic materials for the fabrication of OP (polymethyl methacrylate), silicones, and titanium. The weaknesses of acrylate and silicone are the porousness and roughness of their surfaces. The physical features of these materials support microbial growth on the surface and in the interior of the obturator, from the oral and nasal cavity, which makes maintaining hygiene more difficult. Depending on the general physical condition of the patient and the pathogenicity of microorganisms, local or systemic infections might occur. The advantages of hot polymerized compared to self-polymerized acrylates are that their surface polishes better, which facilitates hygiene maintenance and reduces microbial growth. Due to hypersensitivity of the mucous membranes after radiation therapy, the obturator may also be made of soft acrylate, but in that case, hygiene maintenance is affected because of the porousness of the material. Titanium has good biomechanical properties – it is biocompatible, resistant to corrosion, has little weight, does not cause allergies, and may be easily polished. The weak point of titanium is that it is hardly adapted to the soft tissue structures in the oral cavity, and the cost of such prosthetic work is significantly higher<sup>6</sup>. The weight of the OP may also present a fundamental problem, especially in the case of toothless patients and patients suffering from subtotal edentulism, when the number and the distribution of the remaining teeth do not secure adequate re-

tention<sup>12</sup>. PEEK is a high-performance polymer with good mechanical properties. It is resistant to chemicals and elevated temperatures. It is also applied in medicine as a biocompatible material that may be sterilized. As a material, it is suitable for the fabrication of fixed and mobile prosthetic dental rehabilitation. Prosthetic structures made of this material may sustain forces up to 1,200 N, which is more than sufficient since the maximum bite force for humans is 500 N. The main advantage of this material is its small specific weight (1.32 g/cm<sup>3</sup>) which allows the fabrication of lighter prosthetic structures<sup>7</sup>. The elasticity of this material is like a human bone (4,000 MPa), which, contrary to metal alloys and other materials used in dental prosthetics, significantly reduces occlusal pressure. That is particularly important in implant-bearing prosthetic reconstructions due to lesser pressure on the dental implants<sup>13</sup>. It is resistant to wearing and tearing, as well as to breaking, and there is a weak degradation of the material after longer periods of utilization. It may be easily processed mechanically and highly polished<sup>7</sup>. The material is biocompatible, resistant to plaque, insoluble in water and bodily fluids, may be sterilized, is radiotransparent, does not reflect rays during radiation therapy, and is acceptable from the aesthetic point of view since it is white. Another positive thing about this material is that telescope crowns could be made of it, in combination with various conventional materials or with the same material<sup>7</sup>. One of the main problems of OP is retention and stability. Telescope prostheses ensure good retention, thus resulting in increasing the patient's confidence<sup>14</sup>. Another equally important problem of acrylic OP is the accumulation of bacteria on the surface and infections. A certain number of investigations reveal a substantial accumulation of bacteria and fungi on the surface of an acrylic obturator, which is the source of infection. Infections occur because of bacterial

adhesion on the very surface of the material and their multiplication<sup>15,16</sup>. The nonadhesive surface made of BioHPP reduces the binding potential of cells and proteins on it, thus indirectly reducing the accumulation of the bacteria and the potential for the occurrence of an infection<sup>16</sup>. Recent research shows that the use of this material is suitable for reconstructing the defect of the craniofacial region. Compatible properties and good characteristics of this material show that its application, combined with new 3D printing technologies, will be increasingly used in primary and secondary reconstructive procedures<sup>17-19</sup>. This case report of the patient who had the partial OP based on PEEK, as well as our experience, has revealed a vast number of benefits of this prosthesis in comparison to conventional materials. The advantages are primarily reflected in its functionality due to the decreased weight, good closure and positioning of the prosthesis along the rims, stabilization and retention, satisfactory phonation, and the restoration of the oropharyngeal function. Besides that, the advantages are also reflected in the simpler use and the fact that patients get accustomed more easily to the partial OP and can maintain oral hygiene more easily and effectively.

### Conclusion

With the fabrication of the telescopic partial OP out of PEEK, good retention and stability may be achieved, which will have positive effects on the patient's confidence and comfort. Due to its chemical stability, biocompatibility, and good mechanical properties, oral hygiene maintenance is easier, reducing the potential for secondary infection. The simplicity of the procedure and the relatively low cost of its fabrication ensure functionality, which significantly contributes to the improvement of the patient's quality of life.

### R E F E R E N C E S

1. The glossary of prosthodontic terms. *J Prosthet Dent* 2005; 94(1): 10–92.
2. *de Groot RJ, Rieger JM, Rosenberg AJWP, Merckx MAW, Speksnijder CM*. A pilot study of masticatory function after maxillectomy comparing rehabilitation with an obturator prosthesis and reconstruction with a digitally planned, prefabricated, free, vascularized fibula flap. *J Prosthet Dent* 2020; 124(5): 616–22.
3. *Ye H, Wang Z, Sun Y, Zhou Y*. Fully digital workflow for the design and manufacture of prostheses for maxillectomy defects. *J Prosthet Dent* 2021; 126(2): 257–61.
4. *Sharma AB, Beumer J 3rd*. Reconstruction of maxillary defects: the case for prosthetic rehabilitation. *J Oral Maxillofac Surg* 2005; 63(12): 1770–3.
5. *Kranjčić J, Džakula N, Vojvodić D*. Simplified Prosthetic Rehabilitation of a Patient after Oral Cancer Removal. *Acta Stomatol Croat*. 2016 Sep; 50(3):258-264.
6. *Depprich R, Naujoks C, Lind D, Ommersborn M, Meyer U, Kübler NR, et al*. Evaluation of the quality of life of patients with maxillofacial defects after prosthodontic therapy with obturator prostheses. *Int J Oral Maxillofac Surg* 2011; 40(1): 71–9.
7. *Katzer A, Marquardt H, Westendorf J, Wening JV, von Foerster G*. Polyetheretherketone–cytotoxicity and mutagenicity in vitro. *Biomaterials* 2002; 23(8): 1749–59.
8. *Skirbutis G, Džingutė A, Masiliūnaitė V, Šulcaitė G, Žilinskas J*. A review of PEEK polymer's properties and its use in prosthodontics. *Stomatologija* 2017; 19(1): 19–23.
9. *Chalian VA, Barnett MO*. A new technique for constructing a one-piece hollow obturator after partial maxillectomy. *J Prosthet Dent* 1972; 28(4): 448–53.
10. *Keyf F*. Obturator prostheses for hemimaxillectomy patients. *J Oral Rehabil* 2001; 28(9): 821–9.
11. *Javid NS, Dadmanesh J*. Obturator design for hemimaxillectomy patients. *J Prosthet Dent* 1976; 36(1): 77–81.
12. *Costa-Palau S, Torrents-Nicolas J, Brujau-de Barberà M, Cabratosa-Termes J*. Use of polyetheretherketone in the fabrication of a maxillary obturator prosthesis: a clinical report. *J Prosthet Dent* 2014; 112(3): 680–2.
13. *Papathanasiou I, Kamposiora P, Papavasiliou G, Ferrari M*. The use of PEEK in digital prosthodontics: A narrative review. *BMC Oral Health* 2020; 20(1): 217.
14. *Böttger H*. Das Teleskopsystem in der zahnärztlichen Prothetik. Leipzig: Johann Ambrosius Barth; 1969. (German)
15. *Spencer RC*. Novel methods for the prevention of infection of intravascular devices. *J Hosp Infect* 1999; 43 Suppl: S127–35.
16. *Kyomoto M, Moro T, Konno T, Takadama H, Kawaguchi H, Takatori Y, et al*. Effects of photo-induced graft polymerization of 2-methacryloyloxyethyl phosphorylcholine on physical proper-

- ties of cross-linked polyethylene in artificial hip joints. *J Mater Sci Mater Med* 2007; 18(9): 1809–15.
17. *Alonso-Rodríguez E, Cebrián JL, Nieto MJ, Del Castillo JL, Hernández-Godoy J, Burgueño M.* Polyetheretherketone custom-made implants for craniofacial defects: Report of 14 cases and review of the literature. *J Craniomaxillofac Surg* 2015; 43(7): 1232–8.
18. *Gerbino G, Zavattero E, Zenga F, Bianchi FA, Garzino-Demo P, Berrone S.* Primary and secondary reconstruction of complex craniofacial defects using polyetheretherketone custom-made implants. *J Craniomaxillofac Surg*. 2015; 43(8): 1356–63.
19. *Honigmann P, Sharma N, Okolo B, Popp U, Msallem B, Thieringer FM.* Patient-Specific Surgical Implants Made of 3D Printed PEEK: Material, Technology, and Scope of Surgical Application. *Biomed Res Int* 2018; 2018: 4520636.

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DiMaio VJ. *Forensic Pathology*. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. *The Washington Manual of Medical Therapeutics*, 30th edition. Boston: Lippincott, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. *Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming*; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Aboud S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. *Am J Nurs* [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: <http://www.nursingworld.org/AJN/2002/june/Wawatch.htm>

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U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al.* Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

### Primeri referenci:

*Durović BM*. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

*Balint B*. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

*Mladenović T, Kandolf L, Mijušković ŽP*. Lasers in dermatology. In: *Karadaglić D*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

*Christensen S, Oppacher F*. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

*Abood S*. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: <http://www.nursingworld.org/AJN/2002/june/Wawatch.htm>

### Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

### Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

### Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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